

NI

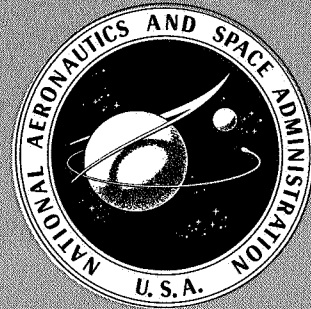
NASA SP-134

THE CLOSED LIFE-SUPPORT SYSTEM

AMES RESEARCH CENTER

Moffett Field, California

April 14-15, 1966



NATIONAL AERONAUTICS AND SPACE ADMINISTRATION

THE CLOSED LIFE-SUPPORT SYSTEM

*Ames Research Center
Moffett Field, California
April 14-15, 1966*

prepared by

Ames Research Center



Scientific and Technical Information Division

OFFICE OF TECHNOLOGY UTILIZATION
NATIONAL AERONAUTICS AND SPACE ADMINISTRATION

1967
Washington, D.C.

FOREWORD

Scientists and engineers have been concerned for many years with the challenging problems inherent in the fabrication of a closed life-support system. Aside from the prospects of difficult engineering hurdles yet to be overcome, it is also clear that enormous gaps exist in our fundamental knowledge about many aspects of this problem. For example, it is impossible at this time even to summarize man's nutritional requirements completely! Virtually nothing is known about human production of contaminants and not much more is available regarding man's tolerance levels to such materials. Biological agents that might be employed to convert human wastes to useful foodstuffs or fuels are themselves incompletely studied, particularly from the point of view of their usefulness as potential "chemosynthesizers." On the other hand, physicochemical procedures to accomplish these end results have barely been initiated.

This Conference, then, is an attempt to examine the present status of some of these problems. Hopefully, lines of future research and development will become clearer as a result of these deliberations and, if this leads to more vigorous and more rigorous experimentation and study, the Conference will surely have been successful.

HAROLD P. KLEIN

Assistant Director for Life Sciences

CONTENTS

	Page
STUDY OF LIFE-SUPPORT SYSTEMS FOR SPACE MISSIONS EXCEEDING ONE YEAR INDURATION	1
G. L. Drake, C. D. King, W. A. Johnson, and E. A. Zuraw General Dynamics/Convair Division	
STUDY OF LIFE-SUPPORT SYSTEMS FOR SPACE MISSIONS EXCEEDING ONE YEAR INDURATION	75
R. B. Jagow and R. S. Thomas, Editors Lockheed Missiles and Space Company, Sunnyvale, Calif.	
PLANS FOR A PROGRAM TO STUDY CLOSED LIFE-SUPPORT SYSTEMS	145
E. Gene Lyman Ames Research Center	
WASTE STABILIZATION IN SPACE ENVIRONMENTS	151
H. G. Schwartz, Jr., and J. E. McKee W. M. Keck Laboratory of Environmental Health Engineering, California Institute of Technology	
THE SPIN-INERTIA CULTURE SYSTEM IN WASTE TREATMENT IN CLOSED SYSTEMS . .	163
Clarence G. Golueke and William J. Oswald Sanitary Engineering Research Laboratory, College of Engineering and School of Public Health, University of California, Berkeley	
DESIGN AND EVALUATION OF CHEMICALLY SYNTHESIZED FOOD FOR LONG SPACE MISSIONS	175
Jacob Shapira Ames Research Center	
PROSPECTUS FOR CHEMICAL SYNTHESIS OF PROTEINACEOUS FOODSTUFFS	189
Sidney W. Fox Institute of Molecular Evolution, University of Miami	
THE EFFECTS OF CONTROLLED ENVIRONMENT ON THE GROWTH OF HYDROGENOMONAS BACTERIA IN CONTINUOUS CULTURES	201
John F. Foster and John H. Litchfield Battelle Memorial Institute, Columbus Laboratories	
ALGAL SYSTEMS FOR BIOLOGICAL FOOD SYNTHESIS	213
C. H. Ward, Rice University, and R. L. Miller, USAF School of Aerospace Medicine	
APPENDIX: ATTENDEES AT THE CONFERENCE	225

STUDY OF LIFE-SUPPORT SYSTEMS FOR SPACE MISSIONS

EXCEEDING ONE YEAR IN DURATION

By G. L. Drake, C. D. King, W. A. Johnson,
and E. A. Zuraw

General Dynamics
Convair Division

INTRODUCTION

This Phase I study is a step toward the goal of providing a closed ecology for manned space missions of long duration. Life support are needed that will operate for periods exceeding one year without resupply. An essential requirement will be the ability to convert human and cabin waste products into useful products such as oxygen, food, and potable water. There is already substantial progress in the closure of the oxygen and water loops (ref. 1) and it is expected that this progress will continue to the point of flight-ready hardware for long-duration missions. Closure of the food-waste loop is considerably less advanced. Therefore, study emphasis was placed on this food-waste closure while work on other life-support subsystems was directed primarily at insuring integration compatibility.

Purpose

The purpose of this study is to verify technical justification and provide planning direction for subsequent phases of the program, namely, Phase II, Research and Development; Phase III, Engineering Design; Phase IV, Construction of a Prototype Closed Ecological System; and Phase V, Evaluation of the Prototype Closed Ecological System. In accordance with this purpose, tasks were performed as follows: (1) The state of development of life support subsystems was reviewed; (2) configurations of closed ecological systems were established; (3) preferred systems were selected, based on estimates of engineering and biological practicality; (4) requirements for research and development to qualify the systems for engineering design were identified; (5) priorities were established and specifications were written for the research and development; and (6) a program plan was prepared for the remaining phases of the program.

Scope

This report summarizes the accomplishments of the first four tasks listed above. Consideration was given to all reasonably applicable concepts for food-waste loop closure within the constraints of space mission use. They were evaluated to a degree of detail commensurate with the fund of technical information. The subsystems were categorized as "biological" and "physico-chemical," and were further divided into those having primarily a food synthesis function and those having waste processing functions.

Biosystems. - The data available on some biological subsystems were sufficient to estimate engineering parameters. These were biosystems based on algae, hydrogen bacteria, higher plants, yeast molds, activated sludge, or anaerobic sludge. The limited data available concerning many other proposed biosystems did not permit evaluations to the same level of detail. The evaluations that could be made did not disclose a preponderance of advantages over those biosystems listed.

Physicochemical subsystems. - Seven different combinations of the steps for the synthesis of carbohydrates from CO₂ and water were studied, and engineering estimates were derived for the two methods for which some process data was available. The chemical synthesis of fats and protein derivatives was reviewed. Waste processes studied included the recovery of potable water from liquid and solid wastes and the oxidation of solid residues.

Method

Technical information was assembled from many sources, including technical literature and government, educational, and industrial organizations. These sources provided information of processes components, analyses, and experimental results, so that the merit of closed ecology could be evaluated. Configurations of the complete closed food-waste loop and supporting subsystems were then developed from competitive subsystems. Engineering parameters were calculated from the reported research data. In many cases, engineering estimates were based on extrapolations from known characteristics of existing systems. The closed configurations selected indicate the preferred channel for subsequent research and development.

SYSTEM MODEL

A spacecraft system model was established to provide ground rules and a suitable frame of reference for evaluating subsystems and the closed configurations. It is solely a tool to help achieve an orderly evaluation, and is not intended or applied in a manner to exclude consideration of any valid concepts. The system model is based on studies of manned planetary missions (ref. 2), and its elements are a mission model, a spacecraft model, a crew model, and a basic performance model for the life support system. Portions that were particularly significant to the evaluations are presented in the following paragraphs.

Mission Model

Mission: Earth-Mars round trip with Mars orbit capture, in support of landing by other vehicles.

Mission periods:	Earth orbit to Mars orbit	230 days
	Planetary capture	50 days
	Mars orbit to Earth orbit	<u>260 days</u>
	Total	540 days
Orbit altitudes:	Earth 325 km	
	Mars 1000 km	

Gravity environment: Extended periods of weightlessness

Solar orientation: Random

Spacecraft Model

Physical configuration: Four cylindrical compartments, with inter-connection or isolation capabilities. Dimensions of each compartment:

Diameter	20 ft (6.10m)
Height	8 ft (2.44 m)

Cabin atmosphere quantities:

Volume, total of four compartments	10,000 ft ³ (283 m ³)
Leakage rate	0

Electrical power supply:

Capacity, normal mode	60 kW
Capacity, emergency mode	10 kW
Power type:	28 VDC and 3-phase ac, 115-208 V, 400 cps
Weight penalty	50 kg/kW

Thermal control:

Heat rejection

Type: Liquid transport to space radiator integral with spacecraft

Temperatures: Radiator inlet, max.	75° C
Radiator outlet	0° C

Weight penalty	15 kg/kW
----------------	----------

Process heat

Type: Liquid transport from a waste heat source in power system

Temperatures: Supply	200° C
Return	100° C

Weight penalty	15 kg/kW
----------------	----------

Crew Model

Number and distribution: Ten men, with normally no more than four in any compartment.

Metabolic criteria, daily averages, per man-day (crew average activity at 150 percent BMR):

O ₂ uptake, g	850
CO ₂ output, g	1000
Water of oxidation, g	315
Water allowance, g	3500
Urine water, g	1500
Evaporative water loss (respiration and perspiration), g	2200
Latent heat, kg-cal	1080
Sensible heat, kg-cal	1500

Life Support System Performance Model

Operating Modes: The life support system (LSS) is not required to operate during boost, but it must withstand g forces of launch and reentry. It will not be required to start automatically but it must operate automatically after it is started. The system will be monitored and maintained by the crew.

Operating lifetime: The LSS shall have an operating lifetime of not less than 600 days. This requirement does not preclude maintenance by the crew.

Atmosphere in crew spaces:

Total pressure, mm Hg	500 ±10
Oxygen partial pressure, mm Hg	160 ±5
Diluent gas	Nitrogen
Carbon dioxide, mm Hg, average	4
Relative humidity, percent	30-60
Toxic gaseous contaminants, max.	0.1 TLV ¹
Temperature (at crew choice), °C	23 ±3

Water management :

Water allowances, g/man-day	
Drinking and in food	3500
Personal sanitation	1500
	<u>5000</u>
Total used	
Waste liquids, g/man-day	
Urine	1500
Humidity condensate	2200
Wash water	1500

Regeneration of consumables: The LSS shall include components for regeneration of water, oxygen, and food from metabolic wastes.

¹Threshold limit value

TECHNICAL DISCUSSION, BIOSYSTEMS

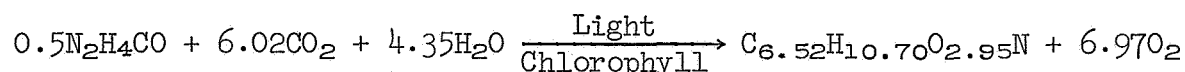
Before individual biosystems were evaluated, the development status of various related biosystems proposed for use in space was surveyed. A qualitative presentation of the survey results is given in table I.

General impressions reached as a consequence of the survey are as follows :

1. Cultural characteristics of organisms presently used in the various biosystems are not considered ideal. Biologists are hopeful that better candidates will be found that will reduce weight, power, and volume required of biosystems.
2. Gas exchange aspects of biosystems have been studied more and developed further than waste utilization or food production aspects.
3. The "raw" biomass generated by any one biosystem does not appear to be acceptable food for humans, physiologically or psychologically. Processing, supplementation and/or compositing will be required to produce an acceptable food.
4. It is unlikely that any of the biosystems can be operated on unsupplemented, unprocessed human wastes except for the activated sludge system that consists of an ill-defined assemblage of different microorganisms.
5. No one biosystem is capable of converting all human wastes quantitatively to food.
6. The fundamental biology and biochemistry of candidate organisms for biosystems are not well understood.
7. Data on material balance of most biosystems are inadequate for precise engineering treatment.
8. Data on long-term, steady-state tests of biosystems are needed to provide a basis for reliability estimates.

Algae

Process description. - An aqueous suspension of unicellular algae can be used to convert CO₂ into algal protoplasm with light as the energy source according to the following equation (refs. 3 and 4):



The process not only results in CO₂ removal, but in the generation of O₂ and algae as well.

TABLE I. - RELATIVE DEVELOPMENT STATUS OF SPACE-APPLIED BIOSYSTEMS

Biosystem	Gas exchange	Waste utilization	Food value
Algae	4	2	2
Higher plants	2	1	1
Lower plants	1	0	0
Activated sludge	2	3	1
Anaerobic sludge	0	1	0
Hydrogen bacteria	1	0	0
Yeast and molds	0	1	0
Animals	0	0	0
Plant or animal tissues	0	0	0

Although a number of algal systems have used different species of algae or⁵ different light sources (incandescent, refs. 4-7; fluorescent, refs. 8-13; and solar), their processes are basically similar. It is essential that they include CO₂, light in the visible range of the electromagnetic spectrum, cooling H₂O, a nitrogen source such as urea, nitrate, or NH₃, and other inorganic salts. Their outputs include oxygen, algae, heat, and trace amounts of organic materials excreted by the algae into the nutrient solution. A material balance and schematic diagram of the system are presented in figures 1 and 2, respectively. In this process, cabin air enriched in CO₂ by human

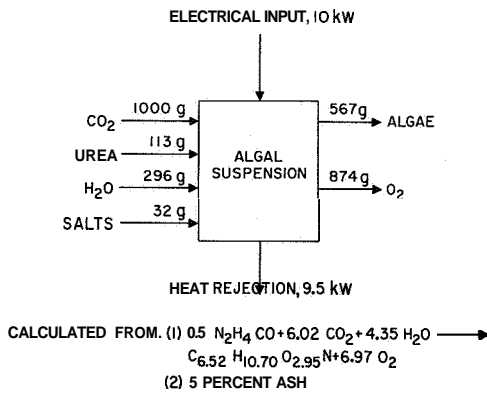


Figure 1.- Algal system material balance; basis: man-day.

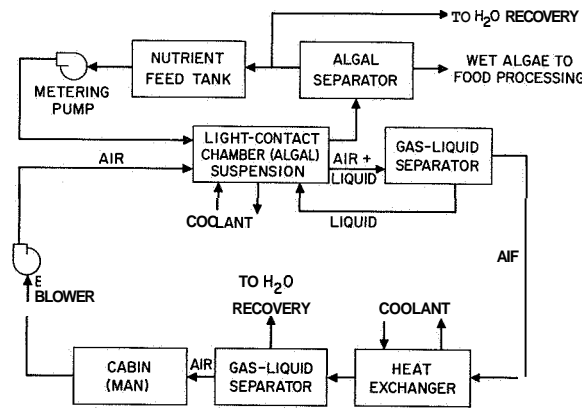


Figure 2.- Schematic diagram of an algal photosynthetic gas exchange system.

respiration is pumped through a light contact chamber containing an aqueous suspension of algae. The function of the light chamber is to contain the algal suspension and provide light for photosynthesis. The photosynthetic activity of the algae causes the removal of CO₂ dissolved in the water and the production of O₂ and more algae. Air enriched in O₂ leaving the light chamber is passed through a gas-liquid separator and excess humidity is removed by a dehumidifier before the air enters the cabin atmosphere.

Algal growth rate and, consequently, the gas exchange rate of the system is governed principally by the quantity and quality of visible light available to the algae (refs. 4, 5, 6, 14, 15). However, insufficient amounts of CO₂, or any required nutrient for that matter, may also limit growth. Steady-state growth can be achieved by fixing the rate at which algal suspension is harvested and nutrient solution is fed (ref. k). Gas exchange performance of a photosynthetic gas exchange system is then governed by the usable light provided each algal cell within the suspension and/or gas transfer achieved by the design characteristics of the light contact chamber. Alternately, the density of the algal suspension can be fixed photometrically (ref. 9). Algae absorb strong visible light but require low intensity light for maximum utilization (refs. 16 and 17). Consequently, thin layers of suspension (<1 cm) are required for efficient light utilization. Inefficiencies in light utilization and spectral characteristics of light sources necessitate cooling the algal suspension to maintain selected growth temperatures.

Mesophilic and thermophilic algae extensively studied are cultured optimally at 25° and 39° C, respectively. Excursions to suboptimal temperatures will result in a decrease in growth rate while an excursion of approximately 10° C beyond optimal temperatures will result in failure of the culture. Temperatures are easily controlled within ±1° C with simple instrumentation, however, and near optimal temperatures are readily maintained. Algae grow optimally at a pH of 6 to 7 but tolerate excursions in pH from 5 to 8. Control of pH is no problem when urea is used as a nitrogen source.

Wastes as algal nutrients.— Untreated wastes are not desirable nutrients for algae. Much of the waste is not in a form available to algae and the high organic content encourages growth of bacteria. The turbidity generated by the bacteria, coupled to the turbid and colored nature of the wastes themselves, can only reduce light utilization efficiency which is extremely important in the culture of algae. Although Chlorella has been grown on urine, growth is not so good as in control media (ref. 17). Untreated feces-urine mixtures have also been fed to coupled activated sludge-algal systems, but comparative performance data of the mixed system versus algae alone are not available for evaluation (ref. 18). There is little question that the by-products of an activated sludge process will support growth of algae. A number of algae (Chlorella, Scenedesmus) grow prolifically in sewage oxidation ponds, and algae are prominent members of the microbial community observed in sewage trickling filters. When the activated sludge by-products are considered in the context of a closed ecosystem, however, investigators have found that supplements of urea (ref. 19) and iron (ref. 20) are required for algae to grow as well as on common media.

Ash derived from incinerated feces and urine has been found to contain all the nutrients required by algae except carbon, nitrogen, and water which were not recovered by the incineration process. With ash concentrations of greater than 1g/L, growth of Chlorella was inhibited (ref. 21). Incineration procedures must be modified to produce a less toxic ash.

Algae as food.- The findings of the various workers in this field can be summarized as follows:

1. Algae are rich in proteins and vitamins but relatively poor in carbohydrate and fat (refs. 4, 22-26).
2. The chemical composition of algae can be varied within limits, depending upon the physical and chemical conditions selected for their culture (ref. 13).
3. The protein quality of algae compares favorably with yeast and other plant proteins. Although analyses suggest that sufficient quantities of amino acids essential for man are available, feeding trials indicate that algal proteins are deficient in sulfur-containing amino acids such as methionine for rats (refs. 24, 26-28).
4. With the use of suitable preparation procedures such as freeze drying or blanching, approximately 80-percent assimilation of algal protein in rats is readily attained (ref. 26).
5. Algae have been incorporated up to 20 percent of the diets of rats with satisfactory weight gains noted (refs. 22, 24).
6. Human subjects have tolerated 50 to 100 g of algae (-20 percent) in their diet. Larger quantities have produced gastrointestinal difficulties (ref. 23).
7. Algae are more acceptable in human diets when admixed in bakery products (refs. 25, 29).

If algae alone are used to balance human respiration, approximately 600 g of dried algae will be produced per man-day. Only 100 g of the algae produced can be utilized as a source of food according to present specifications for human dietary requirements and present evidence of acceptability of algae as a human food source.

In order to use all the alga as food, they would have to be grown under cultural conditions that would yield cells having a composition more nearly matching human dietary requirements and/or yield cells capable of being processed to match human physiological and psychological dietary requirements. The possibilities of doing this have not been investigated to the extent that evaluation is possible. However, if this is done, gas exchange characteristics of the alga will certainly change with changes in internal chemical composition.

If man is not fed all the algae produced, another complication arises with regard to the nutrient salts required to grow the quantity of algae required for gas exchange. Potential elemental outputs of the man on a conventional diet will not match elemental inputs required by the algae (ref. 4).

Advantages. -

1. An algal system performs four useful functions simultaneously: CO₂ removal, O₂ production, synthesis of cellular material requiring minimum modification as a food source, and utilization of some of the waste products of man.
2. Algae can utilize solar energy, if available.
3. Photosynthesis occurs at low pressures and temperatures.
4. The algal cell has built-in, self-regulating, gas exchange control mechanisms.
5. An algal system presents a low health and explosion hazard.
6. Some algae are rugged and grow under wide ranges of environmental conditions. Populations of green algae do not appear to be adversely affected by bacterial, mold, or viral contamination.
7. At least two investigators have claimed, on the basis of qualitative observations, that algae remove some atmospheric contaminants.
8. Measurable toxic contaminants are not produced by Chlorella.
9. Algal systems have been operated reliably for periods up to 6 months.
10. Power demands for algal systems are uniform.
11. Algae can be grown on treated waste effluents of man.

Disadvantages. -

1. The maximum light utilization efficiency of algae is approximately 20 percent.
2. Algal systems dependent on artificial light sources are burdened additionally by a maximum electricity to light conversion efficiency of 20 percent. Approximately 95 percent of energy input must be immediately removed as low grade heat under practical conditions.
3. Most algae tend to foam and/or foul surfaces.
4. Algae will require considerable processing and supplementation to be nutritionally and psychologically acceptable.
5. Algal systems present difficult problems in gas-liquid contacting and gas-liquid, solids-liquid separation under weightless conditions.
6. Harvested algal cells must be processed continuously or properly preserved after harvesting as they are subject to spoilage.

7. Algal systems cannot use feces or urine that has not been processed. The waste output of man will not balance nutrient requirements of the algae unless the man subsists only on the algae.
8. Algal systems exhibit a slow growth response to transient conditions.
9. The protein content of algal cells is extremely high in comparison to man's requirements; the carbohydrate content is low.
10. Algal populations are quickly decimated when invaded by appropriate protozoa.
11. Algal systems will require large volumes because large surface areas are required for maximum utilization of light.
12. Algal systems are heavy because of weight penalties for power and heat rejection and the large volumes of water required.
13. Algae, like all biosystems, are susceptible to ionizing radiation and are adversely affected by toxic components in many common materials of construction.
14. The human respiratory quotient (RQ) and the algal assimilatory quotient (AQ) have not been balanced in long-term tests.

Development status and uncertainties. - Man-scale algal gas exchangers have been designed, fabricated, and tested for short periods of time (hours, refs. 8 and 30). Small units have been operated for periods up to 6 months (refs. 3 and 15). Operational experience under weightless conditions has been minimal. Almost all man-scale units have been designed and tested without flight restrictions in mind. Consequently, only estimates are available for power, weight, and volume. In addition, only gas exchange characteristics of the system have been examined in any detail.

The nutritional and psychological acceptability of algae as food and the development of suitable processing techniques of algae solely as food in a closed ecosystem are two uncertainties in the use of an algal system. Although algae have been incorporated as a portion of the diet of man and rats, total subsistence on algae alone has not been demonstrated. The available evidence indicates that other foods will be required.

A third uncertainty with algal systems involves the degree to which human wastes are converted back to algae. Although algae have been grown on human wastes, bacteria are usually relied on to degrade organic substances and only soluble materials after degradation are utilized by the algae. Insoluble materials not consumable by algae remain. The quantities and type of materials unconsumed, consumed, or excreted by algal systems operated on human wastes have not been accounted for in any study. Complete material and energy balance data of integrated algal systems are needed for engineering evaluation. Accurate input and output measurement of elements such as C, N, O, H, P, S in man and algae are required to determine precisely how closely a balance can be achieved.

A fourth uncertainty with algal systems involves their long-term stability. Biologists have been hampered in tests of this nature by the poor reliability of commercial equipment rather than failure of the algae. Where such failures have been minimal, steady-state cultures of algae have been maintained for periods of months.

Engineering data.- Estimates for the power, weight, and volume of algal photosynthetic gas exchange systems are given in table 11. With changes in

TABLE II. - ESTIMATED POWER, WEIGHT, AND VOLUME REQUIREMENTS FOR A ONE-MAN PHOTOSYNTHETIC GAS EXCHANGER

Reference*	Wet algae (kg)	Culture volume (L)	Illuminated area (m ²)	Total volume (L)	Culture weight (kg)	Power (kW)	O ₂ /hr/kg algae (L)
31	2.3	230	22.3	2260	225	7.5	11
31	1.0	100	---	565	100	4.5	25
31	0.25	25	---	140	24	0.8	150
		143		---		29	
8	3.3	500	29.4			13.4	

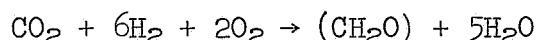
*See also references 32-34.

Basis: 25 L O₂/man-hr

gas exchanger design, it is probable that observed power requirements could be reduced by a factor of 2. Further reductions do not appear probable unless more efficient artificial light sources are developed or algae that utilize light more efficiently are found.

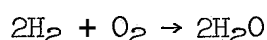
Hydrogen Bacteria

An aqueous suspension of hydrogen bacteria can be used to convert hydrogen and CO₂ into bacterial protoplasm, potentially of human food value, according to the equation (ref. 35):



where (CH₂O) represents cellular matter.

Energy to drive this reaction is derived from the oxidation of hydrogen gas:



Process description. - In this biosynthetic CO₂ reduction system (refs. 36-39), O₂ and H₂ are generated by the electrolysis of water. Part of the O₂ generated and all of the H₂ is fed to an aqueous suspension of Hydrogenomonas. CO₂ removed from the cabin atmosphere and nutrient salts derived

from processed human wastes are also fed simultaneously. The bacteria grow and multiply on the H_2 , CO_2 , and inorganic salts (nutrients) provided, oxidizing H_2 as an energy source for the synthesis of protoplasm. Bacterial cells are harvested at some fixed rate, maintaining a steady-state population and gas uptake rate. Inputs and outputs of the process are estimated in figure 3. A schematic flow diagram is given in figure 4.

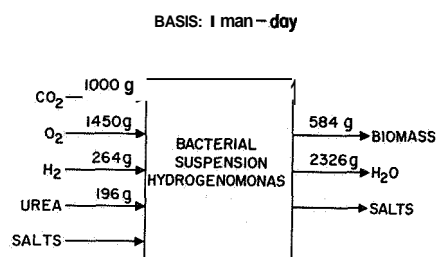


Figure 3.- Partial material balance of a Hydrogenomonas system.

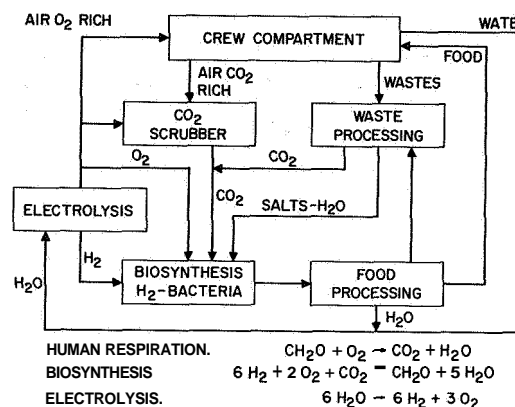


Figure 4.- Schematic flow diagram of a Hydrogenomonas system.

The bacterial growth rate and, consequently, the gas uptake rate are limited generally by nutrient transport rates to each cell and ultimately by metabolic rate limitations imposed by the genetic makeup of the cell to handle available nutrients. Thus, the volume and weight of a bacterial reactor will be governed by the number of bacterial cells per unit volume and the rate at which the cells multiply. The number of bacterial cells per unit volume and the rate at which the cells multiply in turn will be governed by the efficiency with which required nutrients are supplied each individual cell in the population.

The bacterial cells generated are processed for use as food for the crew. Liquid is recycled or sent to water recovery.

Experimental evidence on the optimal growth temperature of Hydrogenomonas eutropha is in conflict (refs. 36 and 40). As with most organisms, however, lethal effects can be expected by excursions to temperatures above the optimal. Temperature excursions below the optimal only result in a reduction in the growth rate. Culture failure due to superoptimal temperatures is easily prevented by suitable instrumentation and selecting an operating temperature approximately $5^{\circ}C$ below that temperature at which adverse effects are observed. Hydrogen bacteria appear to be quite sensitive to oxygen concentration which will have to be suitably monitored and controlled (refs. 35 and 40). The bacteria grow well at a pH ranging from 6.5 to 7.5 (ref. 36). Tolerance to excursions in pH has not been investigated in any detail.

Wastes as nutrients for Hydrogenomonas. Preliminary experiments with hydrogen bacteria have indicated that the organism can be grown on a 1:1 dilution of urine admixed with 1 percent fecal extract in distilled water, but not on a 1 percent fecal solid extract, alone (ref. 36). Comparative rates of growth or yields obtained with urine and a control medium were not determined.

However, gas uptake rates on the wastes (1.3 ml/ml culture/hr) were comparable to those outlined with a control medium (ref. 36).

Stoichiometric relationships of H₂/O₂/CO₂ consumed appear to be affected by the waste mixtures provided the organism. If so, the latter observation will have important ramifications on the feasibility of utilizing unprocessed wastes as nutrients in this biosystem.

Hydrogen bacteria as food.- One determination of the proximate analysis and amino acid content of the organism is presented in table 111. The most

TABLE III.- ANALYSIS OF HYDROGENOMONAS EUTROPHA*

Proximate analysis	Percent	
Moisture	74.55	
Fat	0.44	
Ash	1.73	
Nitrogen	3.02 wet weight basis	
	11.87 dry weight basis	
Protein	18.90 wet weight basis	
(N x 6.25)	74.26 dry weight basis	

Amino acid analysis	Dry weight, percent	Protein, percent
Alanine	4.47	6.02
Arginine	3.41	4.59
Aspartic acid	4.32	5.82
Cystine	0.08	0.11
Glutamic acid	7.67	10.33
Glycine	2.76	3.72
Histidine	0.95	1.28
Isoleucine	2.17	2.92
Leucine	4.04	5.44
Lysine	2.65	3.57
Methionine	1.14	1.54
Phenylalanine	2.20	2.96
Proline	2.06	2.77
Serine	1.80	2.42
Threonine	2.15	2.90
Tryptophane	0.78	1.05
Tyrosine	1.79	2.41
Valine	3.03	4.08

Trace amounts found or methionine sulfoxides, citrulline, α-amino-n-butyric acid, homocitrulline, glucosamine, galactosamine, methionine, sulfoxamine, ethionine, ethanolamine.

*Reference 38.

noteworthy feature of the analysis is the extremely high protein content. All the amino acids essential to humans are present.

Advantages. -

1. The system performs three useful functions simultaneously: CO₂ reduction, synthesis of cellular material requiring minimum modification as a food source, and utilization of urea.
2. Unlike algal systems, converting electricity to light is not required. Electrolysis of water is substituted, which is approximately four or five times more efficient.
3. The system is amenable to automation and control by common engineering process principles.
4. The reaction proceeds at low temperatures and pressures.
5. Unlike algae, reserve bacterial cultures can be carried in a metabolically inactive, freeze-dried state until required for restart.
6. Bacterial cells potentially can be cultured at population densities greater than that of algae.

Disadvantages. -

1. Oxygen to support bacterial biosynthesis must be provided.
2. Potentially explosive gas mixtures must be handled.
3. A bacterial system presents problems in gas-liquid contacting and separation under weightless conditions.
4. The bacterial biomass produced requires processing and supplementation to attain nutritional and psychological acceptability as food. Although high in protein, the bacterial biomass is low in carbohydrate and fat.
5. Harvested bacterial cells must be processed continuously or properly preserved after harvesting to prevent spoilage.
6. Human wastes will very likely have to be processed prior to use as nutrients by the bacteria. Separation and possibly sterilization of waste components may be required. On a conventional diet, a man does not excrete sufficient quantities of urea to support the bacterial biomass required for CO₂ reduction.
7. Bacterial cultures are susceptible to viral attack.

8. Stoichiometric H_2/O_2 gas ratios are dependent on such factors as the type of nitrogen source fed, storage of reserve material, and CO_2 or nitrogen limitations. Unless environmental conditions are kept constant, stoichiometry of gas exchange may change.
9. A pO_2 of 0.40 in the liquid phase of the culture will prevent growth of the culture.
10. Dense bacterial suspensions are inclined to foam when aerated or agitated vigorously for efficient gas-liquid transfer.

Development status and uncertainties. - Man-scale bacterial CO_2 reduction units have not been tested. Small units have only been operated continuously for short periods of time with no operational experience under weightless conditions.

The nutritional and psychological acceptability of bacteria as food and the development of suitable processing techniques are major uncertainties. Hydrogen bacteria are known to produce polymers of β -hydroxybutyric acid as food reserves (up to 60 percent of the dry weight). Utilization by humans is unknown.

The extent to which hydrogen bacteria can utilize human wastes is unknown as is the processing required and quantities actually utilized. Complete material and energy balance data on integrated man-bacterial systems are needed for reliable engineering evaluation.

Data on long-term stability of bacterial cultures under conditions of continuous cultures are also needed for ascertaining mutation, autointoxication, and contamination effects on operational parameters.

Engineering data. - Estimates on power and volume requirements for a one-man bacterial biosynthetic system are given in table IV.

Higher Plants (Hydroponics)

In a hydroponic system, essential mineral elements are supplied as aqueous solutions and plants are grown in the absence of soil (refs. 41-44). Hydroponic systems can be divided into two general types according to the method by which the plants are exposed to the nutrient solution. In the liquid-type hydroponic system, plants are grown in a seed bed that is suspended over a tank filled with a nutrient solution. The plant seeds are sown directly into the mulch where they germinate. Aeration of the nutrient solution is required.

In the semiliquid aggregate type, gravel, sand, vermiculite, or similar aggregate particles are used as an artificial dispersion reservoir for the nutrient solution. The solution is circulated directly through the seed bed, saturating it at intervals of two to seven days. The aggregate moisture level is maintained with water between nutrient applications. Desirable aggregates

TABLE IV.- HYDROGENOMONAS EUTROPHA POWER, VOLUME, AND WEIGHT ESTIMATES
OF A ONE-MAN BIOSYNTHETIC CO₂ REDUCTION SYSTEM

References	37	37	36	36
Culture volume (liter)	50	13	100	75
Cell concentration g/liter (dry)			1	2
CO ₂ uptake/hr/g dry (liter)	0	0	0.04	0.11
Harvest rate (liter/hr)	5	1	33	(3.75)
Power (kW)	1	1	1	1 or < 1
Weight (kg)			45	---
Experimental culture volume		Small scale lab cultures	Small scale lab cultures	Small scale lab cultures
		CO ₂ output of man 22 liter/hr	CO ₂ output of man 31 liter/hr	CO ₂ output of man 22 liter/hr Detention time 20 hr
				CO ₂ output of man 1000 g/day

have a particle size between one-sixteenth inch to three-eighth inch in diameter, resists disintegration or dissolving, are as porous as possible, and do not contain toxic substances or alter pH. Nutrient solutions contain the following mineral elements.

1. Major constituents--nitrogen as urea, NO_3 or NH_4 as a gas, phosphorus as PO_3 or H_2PO_4 , sulfur as sulfate, and potassium, calcium, and magnesium as free ions.
2. Minor constituents--iron, manganese, boron, copper, zinc, and molybdenum.

Nutrient flow rates must be regulated to assure adequate aeration through the aggregate.

The environmental conditions (temperature, humidity, and atmosphere gaseous composition) required for plant growth are quite similar to those required by the crew. Little is known of the effect of reduced or zero gravity upon plant growth.

From an efficiency and practicality standpoint, the initial hydroponic system probably will utilize only a few plants. Several plants that appear suited to hydroponic farming are soy beans, tomatoes, and sweet potatoes. The advantages of these plants are their botanical compatibility, their individual nutrient value, and their adaptability to processing in a variety of forms. Other plants investigated include sugar cane, corn, sunflower, endive, Chinese cabbage, and tampaia.

Process description.— A schematic diagram describing a conceptual space-craft hydroponic system is presented in figure 5. The hydroponic system operates in the following fashion.

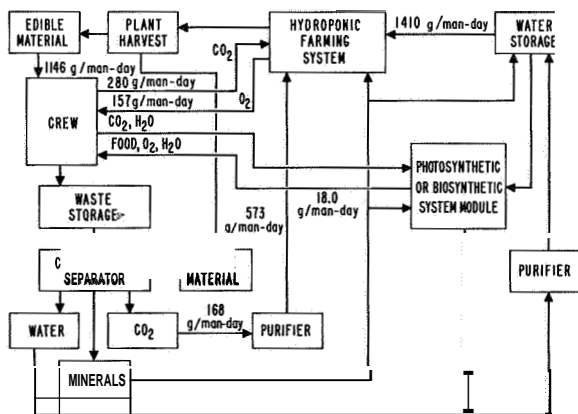


Figure 5.- Schematic diagram of an integrated hydroponic farming system.

At the beginning of each growth cycle (average growth cycle ~90 days) the plant seeds are sown in the aggregate substrate. The substrate is kept moist prior to and following seed germination and throughout the growth cycle of the plant, the aggregate is periodically (every 2 to 7 days) saturated with a nutrient solution. As the plant crop matures, it is harvested and processed to obtain the edible portion. The hydroponically grown food is consumed by the crew as a part of their diet. A portion of the CO_2 produced by the crew is fed to the hydroponic system to maintain its CO_2 level and the oxygen liberated by the hydroponic

plants is recirculated to the crew compartments. Crew waste products (feces, urine, garbage, humidity condensate, wash water, etc.) are processed by physicochemical means to obtain potable water, minerals, and CO_2 . The CO_2 is

recycled back to the hydroponic system. The water obtained is routed to storage where a portion is eventually used for the hydroponic nutrient solution or for maintaining the hydroponic aggregate moisture level. A material balance for a typical hydroponic system is presented in figure 6. It should

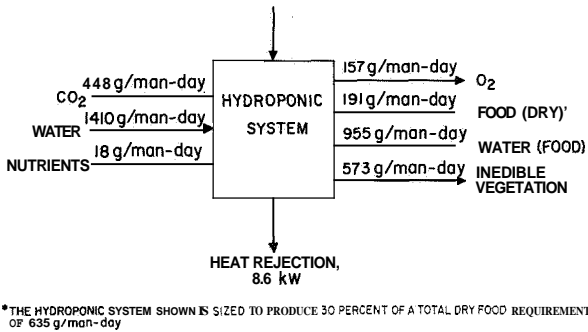


Figure 6. - Hydroponic system material balance.

be noted that the numerical values presented in the material balance as well as in the system schematic are estimates only and do not represent experimental values.

Wastes as nutrients for higher plants. - Unprocessed human wastes cannot be used by higher plants as nutrients. In agriculture, the metabolic activities of a vast flora of microorganisms in soil are relied upon to transform wastes into available nutrients. The wastes added to the soil, moreover, constitute a minor fraction of the total soil mass, and degradation products are subject to the continual leaching action of rain-

fall which prevents a toxic accumulation of any little-utilized material such as NaCl. If the complex interactions and succession of microorganisms in soil are considered, the time required for complete conversion of waste in soil to nutrients suitable for higher plants presumably must take months.

Initial experiments with activated sludge waste effluent and three species of higher plants (Chinese cabbage, tampala, and endive) indicate that the effluent does not serve as a good source of nutrients for the plants (ref. 42). An excess of sodium and chloride is apparent. Growth of Chinese cabbage was improved considerably by the addition of trace amounts of copper. Although copper was present in the effluent according to elemental analysis, it was not present in a form available to the plant. The same problem may exist for other required elements.

Dried sludge from an activated sludge culture has been successfully used in the culture of lettuce (ref. 45). Microbial degradation of the sludge to plant nutrients is presumably required, however, for higher plant utilization.

Higher plants as food. - Since higher plant products are a common part of human diets, this type of food should be acceptable with minimal processing. Proximate analyses of crop plants, such as sugar cane, corn, sunflower, soy beans, etc., can be found in agricultural handbooks. Proximate analyses for endive, tampala, and Chinese cabbage show that the protein and carbohydrate contents of these plants approximate normal human dietary requirements (ref. 46).

Advantages. -

1. Food from higher plants is highly acceptable from a psychological and physiological standpoint.

2. CO_2 is absorbed and O_2 is produced as a part of the photosynthetic reaction.

Disadvantages. -

1. The higher plant system has a high fixed weight, volume, and power requirement .
2. The system has high heat rejection requirements.
3. The ratio of unedible to edible parts of some plants is high.
4. System operation is difficult under zero gravity.
5. Plant growth characteristics under zero gravity are unknown.
6. Food production rate is slow and periodic, and the food requires processing for preservation.
7. Plants are sensitive to environmental conditions.
8. Large amounts of transpired water must be handled.

Development status and uncertainties. - Development of hydroponics for space use is still in the early stages. Conclusive data are lacking in the areas of photosynthetic efficiency, sensitivity to various environments, and operation and growth under zero gravity. Some vegetables, such as tomatoes, are currently being-grown hydroponically for commercial markets. Further definition of ancillary equipment requirements is needed.

Engineering data.- The weight, volume, and power requirements of a conceptual system to satisfy 30 percent of a man's food requirement are estimated as follows:

1. Power: 10.8 kW
2. Volume: 21 ft³
3. Surface area: 42 ft²

Other Food-Producing Biosystems

Other biosystems have been proposed for food production and/or gas exchange in closed ecological systems. The limited data available concerning these systems does not permit a detailed evaluation. They do not show any outstanding advantages over biosystems already discussed.

One of the biosystems proposed utilizes members of the lower plants such as Spirodella (duckweeds) which would perform the same functions as algae or higher plants (refs. 47, 48). Lower plants have almost all the disadvantages

of the former two systems and no outstanding advantages. Advocates of the biosystem stress that this system presents simpler gas transfer operations under conditions of zero gravity.

The use of herbivorous invertebrate animals, such as *Daphnia* and *Artemia*, has also been proposed as links in a food chain consisting of algae-invertebrates-man. From an energetics point of view, the use of intermediates in a food chain is undesirable because the ecosystem must be penalized in terms of weight and volume in proportion to the inefficiencies in food production of the intermediate. Furthermore, operations and control of the ecosystem become more complex with each additional subsystem. Biosystems dependent on herbivorous fish such as *Talapia* and animals such as chickens and rabbits present the same serious disadvantages already mentioned.

Some research has also been conducted on the culture of plant tissues as a source of food (refs. 49-51). The complex nutrient medium required for tissue culture, tissue susceptibility to microbial contamination, and tissue growth habit and growth rate appear to be major roadblocks to the use of plant tissues as a source of food. The same reasoning holds for animal tissue culture.

Waste Processing Biosystems

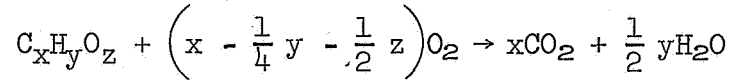
Little research has been devoted to waste processing techniques with the specific objective of producing nutrients for biosystems. For quantitative work with biological systems, biologists select defined media in which the concentration of each component is known, as the use of poorly defined substances often produces unexplainable perturbations in the system under study. Urine and feces are prime examples of poorly defined substances and yet are the substances that must be studied to determine the degree of waste-food closure possible in a closed ecology. Although the list of known components of feces and urine derived from conventional diets is rather imposing (refs. 48, 49), feces and urine have not been completely characterized, especially, the organic components in feces. The components in both wastes, moreover, vary widely depending on diet and donor and it is very difficult to collect and preserve material of reasonable uniformity for study.

Activated Sludge

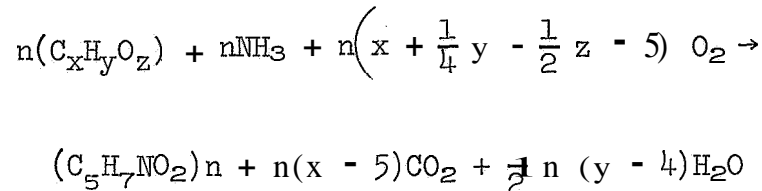
The activated sludge process allows recovery of water from feces and urine and/or the degradation of soluble organic wastes into compounds utilizable in photosynthetic systems (refs. 52-55). Organic matter in the waste is oxidized by encouraging growth of a mixed flora of aerobic microorganisms. Although most of the soluble organic matter is oxidized to CO_2 and water, a by-product of the process is microbial cellular material. Furthermore, a residual quantity of organic matter refractive to microbial degradation remains.

Process description. Equations for the process are as follows (ref. 52) :

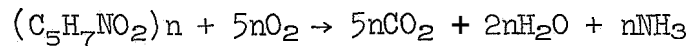
Waste oxidation:



Biomass synthesis:



Biomass oxidation:



Essential inputs to the process include O_2 and cooling water. Outputs of the process include CO_2 , bacterial cells, refractive organic matter, and heat as shown in the schematic flow diagram (fig. 7) and by the material balance (fig. 8). In this process, solid wastes are macerated and blended

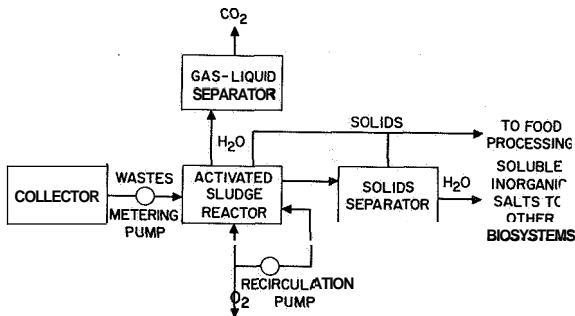


Figure 7.- Schematic diagram of an activated sludge waste treatment system.

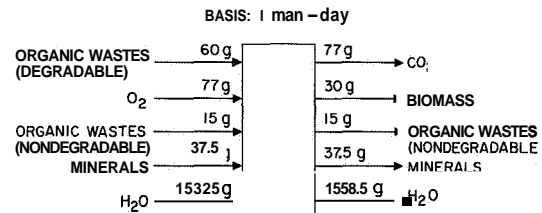


Figure 8.- Material balance, activated sludge.

with urine and personal hygiene waste water to form a finely dispersed suspension. The waste is pumped continuously or semicontinuously to a reactor containing a microbial suspension that must be supplied oxygen. On demand of a sensor in the aeration tank O_2 is added to the bacterial suspension. Gas and liquid are mixed by recirculating the suspension within the reactor. Since CO_2 is produced by the system, this gas, along with some O_2 , is vented through a gas-liquid separator. Microbial suspension is periodically or continuously discharged to a solids separator. The microbial biomass is returned to the aeration tank and the treated waste water is pumped to other biosystems as a source of nutrients.

Microbial growth rates and consequently the waste processing rate of the system is governed principally by O_2 available to individual microbial cells. However, it should be recognized that insufficient quantities of any required microbial nutrient can limit growth. Also, the physiologically desirable temperature range (20° to 40° C) and pH(7-8) must be maintained. Steady-state growth conditions can be achieved by fixing the rate at which wastes of uniform composition are fed and microbial suspension is harvested.

Advantages. -

1. An activated sludge system can transform organic wastes into nutrients of known benefit to photosynthetic systems.
2. The mixed microbial flora of an activated sludge system can perform the waste treatment function under a wide range of environmental conditions and can oxidize a wide spectrum of wastes.
3. Activated sludge systems have operated reliably for periods of years under earthbound conditions.
4. Large quantities of noxious gases are not generated by the process provided a sufficient quantity of O_2 is available for microbial oxidation.
5. The activated sludge process proceeds at low temperature and pressures.
6. Volatile wastes are stabilized by the process that simplifies eventual water recovery operations.

Disadvantages. -

1. An activated sludge system presents difficult problems in gas-liquid contacting and gas-liquid, solid-liquid separation under weightless conditions.
2. If suboptimum quantities of O_2 are provided, undesirable volatile compounds (e.g., NH_3) or partially oxidized compounds (e.g., NO_2) may be generated.
3. Complete mineralization of organic matter is not accomplished by the process in a reasonable time period.
4. The process is relatively inflexible to transient conditions.
5. Failure to supply O_2 to the system for extended periods of time will encourage the establishment of an anaerobic flora of microbes and the production of undesirable end products (e.g., CH_4 , H_2S).

Development status and uncertainties. Activated sludge systems have been used for years by municipalities for the treatment of domestic and/or a wide variety of industrial wastes. Experience with the process has largely been with very dilute wastes (< 1.0 percent solids), while a process capable of handling more concentrated wastes is required for space. A spacecraft scale unit handling concentrated wastes was tested for 30 days in the MESA experiment with less than acceptable results.

There is some experimental evidence that wastes at high solids concentration can be handled by the activated sludge process. Uncertainties involve steady-state processing rates and resultant material balances (refs. 45, 56, and 57). Effluents from the process have been shown to be suitable for growing algae, but supplementation is generally required.

Another uncertainty with the activated sludge system involves the disposition of suspended organic matter. Suspended organic matter in conventional waste treatment is shunted to a different microbial system. To recycle matter in a closed ecosystem, the bacterial biomass generated and the organic matter refractive to rapid microbial degradation must be oxidized to CO₂, H₂O, and salts. It should be noted that the microbial biomass has been used as a feed supplement for chickens and pigs and, therefore, may have some human food value if processed. Preliminary experiments have also shown that lettuce can be grown on suspended solids from the process.

More detailed data are needed on the long-term stability and management of the activated sludge process. The stability of an activated sludge process depends upon "steady-state" growth of microorganisms. Steady-state growth, in turn, depends on genetic integrity and a relatively constant physical and chemical environment. This presupposes a constant feed rate of wastes of reasonably uniform composition.

The effects of mutations should be minimal in a continuous process with a mixed microbial flora. Mutants generally exhibit lower growth rates than the parent strain and will be washed from the system. A continuous process, in fact, will favor microorganisms that can most effectively subsist on the waste. The mixed flora of activated sludge also reduces the probability of process failure due to bacteriophage. It is likely that other species competing for similar substrates and resistant to a particular phage will quickly supersede a phage-infected species.

Engineering data.- Rough estimates of weight, volume, and power are based on an input of 2.5 liters/man-day, which includes feces, urine, and about one liter of other waste liquid. Flow through the activated sludge reactor will be slightly higher, as it will include recycle flows from the solids separator and from the gas-liquid separator. It is estimated that a liquid volume of 6 liters/man will allow a retention time of two days. The following estimates were made for the total subsystem, including reactor vessel, separators, pump, and mixer, but excluding waste collectors and temporary holding components. The requirements per man are:

1. Volume: 13 liters
2. Weight: 12 kg
3. Power: 50 W

Anaerobic Sludge Digestion

Anaerobic sludge digestion is a biological process in which a mixed flora of microorganisms is used to decompose organic matter in the absence of molecular O_2 . Two groups of microorganisms are believed to be responsible for digestion. One group reduces complex organic compounds to simple acids and alcohols while the second group metabolizes the organic acids and alcohols mostly to methane and CO_2 . Oxidations are performed through the use of nitrates, sulfates, and carbonates as oxidizing agents. The oxidation products formed include CO_2 and water, whereas the reduction products are methane, reduced organic compounds and small amounts of ammonia, molecular nitrogen, and hydrogen sulfide. Although most of the organic matter (90 percent maximum) is reduced to methane, CO_2 and water, a residue of organic matter admixed with microbial cells remain (ref. 58).

Process description.- As shown in the schematic flow diagram (fig. 9) and material balance (fig. 10), inputs to the process include organic matter and

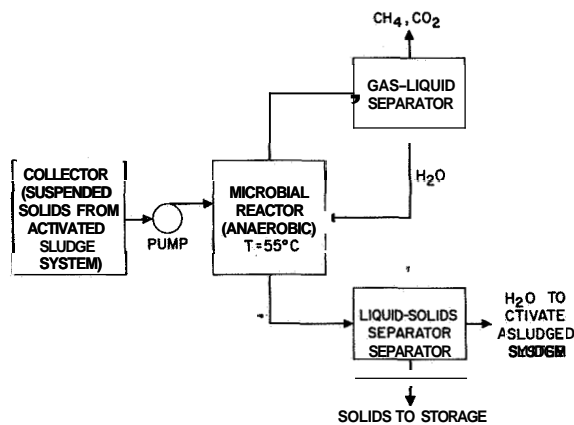
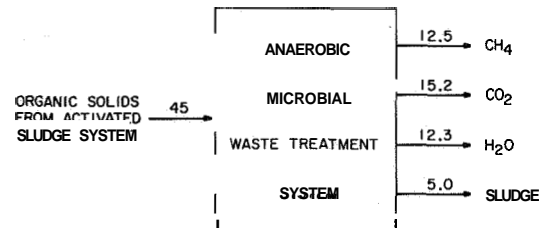


Figure 9.- Schematic diagram of an anaerobic microbial waste treatment system.



BASIS:

1. 90 PERCENT REDUCTION OF ORGANIC MATTER POSSIBLE WITH ANAEROBIC SYSTEM
2. DIGESTION OF 1g OF ORGANIC MATTER PRODUCES .625 liter OF METHANE (70 PERCENT) AND CO_2 (30 PERCENT)
3. WITH HIGH DIGESTION RATE, A DETENTION TIME OF 10 days IS POSSIBLE
4. LOADING OF 11 g ORGANIC MATTER/liter-day POSSIBLE
5. ONE gram OF WET SLUDGE SOLIDS OCCUPIES A VOLUME OF 82 ml

Figure 10.- Material balance, anaerobic microbial waste treatment system; basis: g/man-day .

heat. Outputs of the process include methane, CO_2 , and microbial cells admixed with organic matter refractory to degradation.

In the process shown, organic matter from an activated sludge subsystem is pumped continuously or semicontinuously to a reactor containing anaerobic microorganisms maintained at $55^{\circ}C$. The reactor is operated on a minimum waste detention period of 10 days and is mixed continuously. Gases are vented through a gas-liquid separator, while solids from the reactor are removed

through a solids separator. Water derived from the process is recycled through the activated sludge subsystem or is pumped to water recovery.

The waste processing rate of an anaerobic microbial waste treatment system is low. Detention times of 5 to 30 days are required depending on temperature and organic loads imposed on the system. An anaerobic system, however, can handle organic solids concentration up to approximately 50 percent. Degradation occurs most rapidly at a reactor temperature of approximately 55° C, but degradation proceeds at somewhat reduced rates at temperatures ranging from 20° to 60° C. A stable degradation rate depends on a fixed temperature, a neutral pH, and uniform, nontoxic wastes and loading rates (15 g of organic matter/liter/day maximum).

Advantages. -

1. Microbial anaerobic waste digestion complements aerobic waste digestion by further transforming organic wastes into compounds suitable for recycle.
2. The process takes place at low temperatures and pressures.
3. Wastes containing 2 to 50 percent solids can be handled by the process. (Normal range 2 to 30 percent)
4. Oxygen is not required.

Disadvantages. -

1. Anaerobic microbial waste treatment is slow. (Detention periods of 30 days are commonly used by municipalities.)
2. Methane is produced as a by-product which may require separation from CO₂ and further processing before it can be recycled.
3. Digestion is incomplete, necessitating further handling of solids.
4. Trace amounts of gases such as H₂, CO, and H₂S are produced by the process.
5. Pathogenic microorganisms survive the anaerobic waste treatment process.
6. Accidental admission of O₂ adversely affects the process.
7. Difficult problems in gas-liquid contacting and gas-liquid, solid-liquid separation under weightlessness are presented.
8. The process is relatively inflexible to transient conditions.

9. Volume and weight requirement will be higher than activated sludge waste treatment because throughput rates must be lower to attain degradation.

Development status and uncertainties. Anaerobic sludge digestors have been used for years by municipalities for the digestion of suspended organic solids obtained from settling basins in waste treatment plants. The fundamentals of the process are not well understood and operations have largely been conducted on an empirical basis. There has been very little research in applying this process to waste treatment in space.

A number of uncertainties associated with anaerobic digestion must be investigated to allow proper evaluation of this process. One uncertainty involves steady-state processing rates and resultant material balances attainable. For example, estimates or observations on detention times required range from 5 to 30 days and loading rates range from 0.5 to 15 g of volatile matter/liter/day. Experimental data should be obtained that show that the process can handle anticipated wastes at high concentrations over an extended time period with reasonable processing time and that a high degree of digestion is achieved.

There is also some question as to the disposition of the by-products produced by the process, namely, methane, sludge solids, and effluent water. The methane conceivably can be used in chemosynthesis of sugars or to maintain reactor temperature by combustion. Sludge solids from the reactor will be difficult to handle and are not likely nutrients for any of the biosystems. The sludge solids presumably could be stored or completely oxidized by physical chemical techniques. Effluent water containing dissolved solids probably can be handled by recycling through the activated sludge and other biosystems before water recovery.

Engineering data. Rough estimates of weight, volume, and power for the anaerobic system are based on a sludge input of 3.5 liters/man-day derived from the activated sludge process. It is estimated that a liquid volume of 40 liters/man will allow a detention time of 10 days. Estimates which follow were made for the total subsystem, including reactor vessel, separators, pump and mixer, but excluding waste collector and temporary holding components. Requirements per man are:

1. Volume: 42 liters
2. Weight: 42 kg
3. Power: 127 W

Yeasts and Molds

Fungi that conceivably could be used in closed ecological systems include molds, yeasts, and mushrooms. Potential ecological uses of fungi are:

1. A portion of the diet may be provided by certain molds and mushrooms.

2. Molds and yeasts may be used to stabilize urine and fecal waste while producing microbial cell substance.
3. Certain species of yeasts might be useful for separating edible and inedible sugars produced synthetically, if this proves to be necessary.

Process description. - Two basic fungal systems have been considered in this study. The first utilizes mushrooms as part of the diet (refs. 59-61). The mushrooms are grown on a synthetic compost made up of material high in cellulose and nutrients from urine and fecal wastes. The compost is conditioned by repeated wetting, aeration, and mixing until it is broken down by the organisms present. After the compost is added to the growing beds, it is pasteurized by raising bed temperature to 135° to 140° F for 24 hours. The temperature then is reduced to 75° to 80° F and the propagating material or spawn is added to the moist bed. Then a thin layer of a peat-like soil is spread over the bed and kept moist. Three to four weeks later, mushrooms appear and the temperature is lowered to 60° F. The humidity of the air is maintained at 70 to 80 percent during the growing period. Mushroom production may continue over a period of two or three months.

The second fungal system considered in this study involves the use of molds in the waste processing system to stabilize urine, to produce microbial cell substances, and to oxidize cellulose. A variety of molds and a few yeasts have been examined for conversion of urine and/or feces into potential food (refs. 62-64). Of approximately 30 species of molds examined, Rhizopus delemar and members of the genus Aspergillus were found to grow well on media containing feces or urine. The yeast Torula utilis also grew favorably on media containing urine. In all these studies, however, media containing waste products were supplemented with large amounts of glucose (-5 percent), and no data were presented for yields from wastes in the absence of glucose. Neither urine nor feces contain significant quantities of glucose or products easily converted to utilizable sugars. In view of the glucose requirement, an incubation period of approximately 72 hours and incomplete waste conversions, the use of molds or yeasts does not appear suitable for waste utilization in a closed ecosystem. The same arguments hold for studies with other species of molds. (ref. 65).

Wastes as nutrients for yeasts and molds. - Molds and yeasts exhibit a heterotrophic mode of nutrition, that is, they utilize an organic source of carbon to obtain energy. The organic carbon source is generally supplied as a simple sugar such as a glucose. In the presence of adequate amounts of O₂, molds oxidase the carbon source to CO₂ and H₂O and increase in mass. Yeasts produce the same end product as molds if supplied sufficient O₂. In the absence of O₂, yeasts ferment the carbon source producing ethylalcohol, CO₂, and H₂O and increase in mass. From an energetics point of view, fermentative metabolism is less efficient than aerobic respiration.

Yeast and molds as food. - Preliminary work on the nutritional properties of the organisms has indicated that the material is not suitable as a food for

rodents. Proximate analyses of yeasts and molds indicate generally high protein contents similar to algae and hydrogen bacteria (ref. 66).

Advantages. -

1. Fungi offer a means for oxidizing cellulose.
2. Fungi provide for a variety in the diet of the crew.
3. Fungi may be grown on some components present in waste products.
4. Power requirements are low (light not required).

Disadvantages. -

1. Nutritive and caloric value of fungi is low (mushrooms have a caloric value of 40 cal/lb).
2. Useful fungi may become infested and harmed by other types of fungi.
3. Some fungal systems, such as mushrooms, are not amenable to zero gravity.
4. Weight and volume requirements are high for some fungal systems (mushrooms).
5. Fungal systems consume oxygen and produce CO₂.

Development status and uncertainties. - Mushrooms are grown commercially on synthetic bed material largely on an empirical basis. The process is not readily adaptable to zero gravity operation. The use of yeasts or molds for waste treatment or food production as such also does not appear promising because of requirements for large amounts of simple sugars as nutrients.

Engineering data.- A rough weight estimate for a mushroom culturing system is $351-0x \text{ lb/man}$ where x is the weight of mushrooms per man-day used in diet. A volume of $30x \text{ ft}^3/\text{man}$ is anticipated. Power required for fungal systems is limited to that required by ancillary equipment only. No weight estimates are available for other fungal systems.

Other Waste Treatment Biosystems

There are other biosystems that conceivably could be used for waste treatment in a closed ecological system. Waste treatment by means of trickling filters, oxidation ponds, and composting, for example, all depend on biological agents for waste stabilization.

The trickling filter process is essentially the same as the activated sludge process except for the mode of aeration and is closely allied to the manner in which wastes are stabilized in rivers. In this process, dissolved

organic wastes are passed over a stationary film of microorganism formed on coarse aggregate for oxidation rather than being intimately mixed with the microorganisms as in the activated sludge process. Oxygen is provided by diffusion from the atmosphere. Oxidation ponds possess the characteristics of both activated sludge and anaerobic sludge digestion processes. Dissolved and colloidal organic matter is degraded by aerobic microbes suspended in the pond water. Oxygen is provided by photosynthesis of algae and by diffusion from the atmosphere. Suspended matter sinking to the bottom of the pond is degraded by anaerobic microorganisms for the most part. Composting is dependent on a mixed flora of aerobic microorganism and is generally used to process garbage and other wastes of relatively high solids content.

None of these processes offer obvious advantages for processing waste. Furthermore, no one biological system or combination of biological systems completely reduces waste within reasonable time periods. All of these processes introduce additional problems in engineering design and system operation.

TECHNICAL DISCUSSION, PHYSICOCHEMICAL SUBSYSTEMS

One of the major problems in utilizing biosystems for food production is obtaining the proper dietary balance between the quantities of protein, carbohydrate, and fat. The biological systems generally considered for food synthesis produce biomass disproportionately high in protein. It thus becomes important to find techniques for providing the carbohydrates and fats required.

Carbohydrate Synthesis

Seven methods were investigated for generating carbohydrates from metabolic waste products. All utilize the same technique for producing sugars from a formaldehyde-water solution. They differ basically in their method of producing formaldehyde.

Process descriptions.- In the first method, shown in figure 11, concentrated CO_2 is electrolyzed to CO and O_2 by passage through a solid electrolyte cell subsystem (ref. 67). The CO and unelectrolyzed CO_2 then pass through a regenerative heat exchanger where they are cooled. These gases are compressed and expanded in a liquefier-separator where the CO_2 is removed and recycled back to the CO_2 electrolysis unit. An alternate method of CO_2 separation might consist of CO_2 removal by passage of the gases through a molecular sieve that preferentially adsorbs CO_2 .

The CO as well as some H_2 is passed to a catalytic reactor where they react to form methanol. This reaction takes place at 250°C and about 300 atmospheres during commercial methanol production. Catalysts that may be used include a zinc-oxide base catalyst with a chromium oxide promoter and a copper-oxide base catalyst with a zinc-oxide promoter. The zinc-oxide base

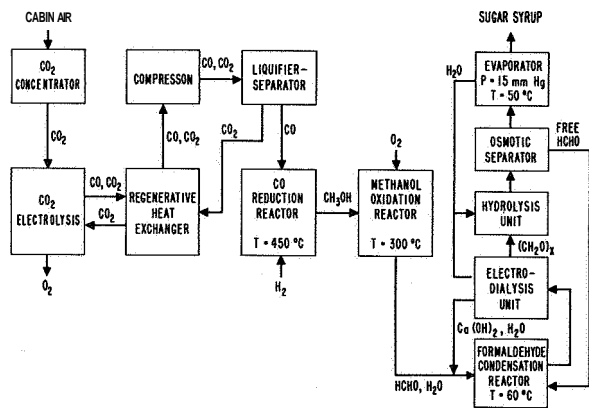
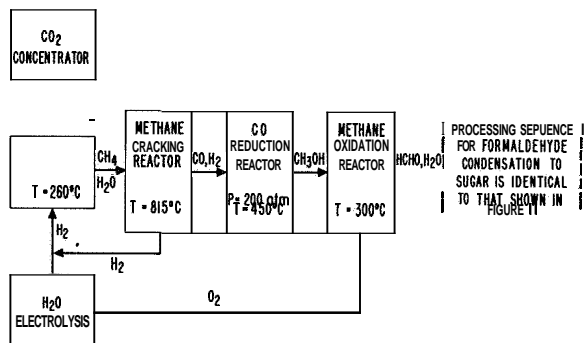


Figure 11.- Carbohydrate synthesis, method 1.

catalyst is generally preferred because of its long active life and resistance to poisoning (ref. 68). This process is of the recycle type in which 12 to 15 percent of the gas feed is converted per pass. The methanol produced is then oxidized in the presence of a silver or molybdenum oxide-iron oxide catalyst at approximately 300° C to form formaldehyde and H₂O. Yields up to 92 percent have been obtained (ref. 69). This "formalin" solution, in the presence of a calcium hydroxide catalyst and at about 60° C, is condensed to form formose sugars. At the completion of the condensation process, the hydroxide is removed from the sugar solution for recycling by electro dialysis.

It has been suggested (ref. 70) that relatively large quantities of semiacetals may be present in the formose sugars. These semiacetals when ingested are broken down by the acidic juices, and release relatively large amounts of formaldehyde and cause strong toxic effects. To eliminate this toxic effect, the sugar acetals are split by hydrolysis resulting in sugar and free formaldehyde. The free formaldehyde is then removed from the sugar solution through a permeable membrane by inverse osmosis under pressure. The free formaldehyde is recycled to the condensation reactor to form more sugars. The sugars are then concentrated by passage through a flash evaporator at about 50° C and a low pressure of 15 mm Hg. Water extracted in the evaporator is condensed and portions returned to the hydrolysis unit and the electrolysis unit.

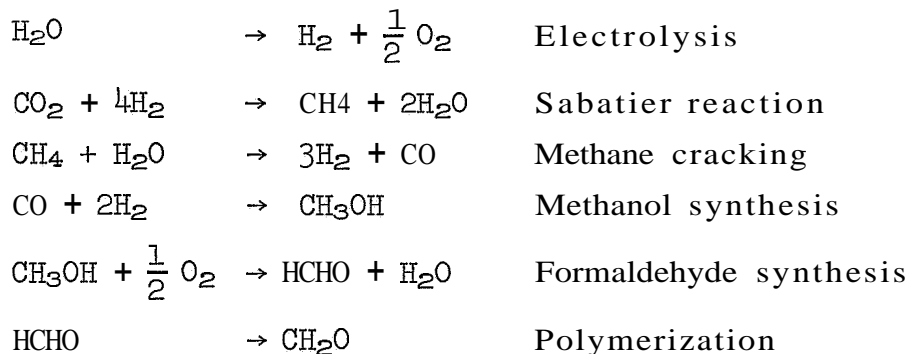


lyst at about 500° C. The hydrogen is

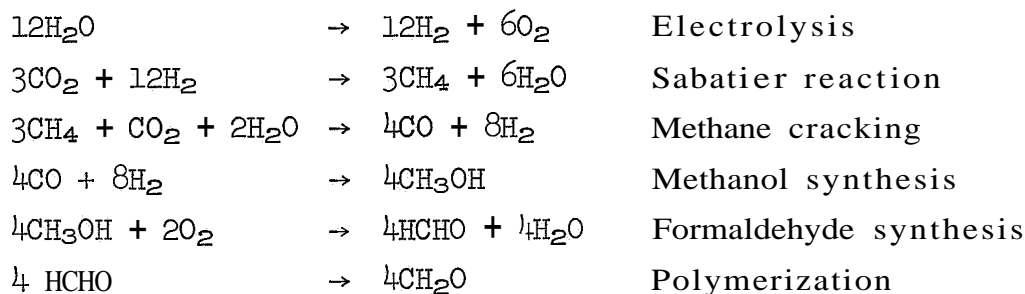
cient (~90-95 percent). The amount of catalyst consumed during the reaction is negligible. The reaction products,

then pass to a catalytic reactor where they are reduced to CO and hydrogen in

recycled to the Sabatier reactor. The remaining hydrogen and CO are then processed in a manner identical with that used in method 1 to obtain the concentrated sugar syrup. Thus, the reaction sequence involved is:



A disadvantage of this method is that it requires the separation of hydrogen from the reaction products of the methane cracking reactor to achieve the proper CO/H₂ ratio for methanol synthesis. An alternate procedure would be to add CO₂ to the gas mixture entering the methane cracking reactor, thus arriving at reaction products with the proper CO/H₂ ratio. This reaction sequence is shown below:



In the third method (fig. 13), methane is produced from CO₂ and H₂ by the Sabatier process. The methane is then converted directly to formaldehyde by partial oxidation with air or oxygen.

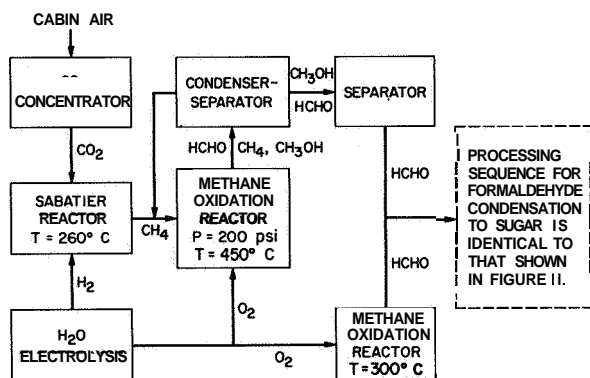


Figure 13.- Carbohydrate synthesis, method 3.

This is an exothermic reaction that takes place at moderate pressure (100-300 psi) and 450° C and utilizes aluminum phosphate and metal oxide catalysts. The methane is premixed with oxygen and passed through a heat exchanger, the heat source being the recycled hot products of the CH₄ oxidation reactor where a mixture of CH₄, CH₃OH, and HCHO is formed. These gases go through the preheater to the condenser. The noncondensable gases are recycled to the raw gas stream. Condensed CH₃OH and HCHO are separated with the HCHO passing to the HCHO

condensation reactor and the CH_3OH directed to a CH_3OH oxidation reactor where additional formaldehyde is formed. The formaldehyde is then processed to obtain sugars in a manner similar to method 1.

The advantages of this method over that of method 2 are:

1. The reaction step, methane cracking, is eliminated.
2. Each of the major steps in the reaction sequence is exothermic and, hence, requires little or no process energy.
3. Processing steps do not require excessively high pressures and, temperatures to achieve high yields.

In the fourth method (fig. 14), concentrated CO_2 is used to form methanol directly. The methanol is then used to synthesize formaldehyde and sugars in a manner similar to method 1. The reaction sequence is shown below:

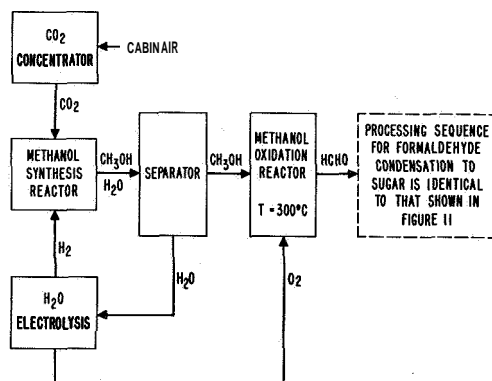
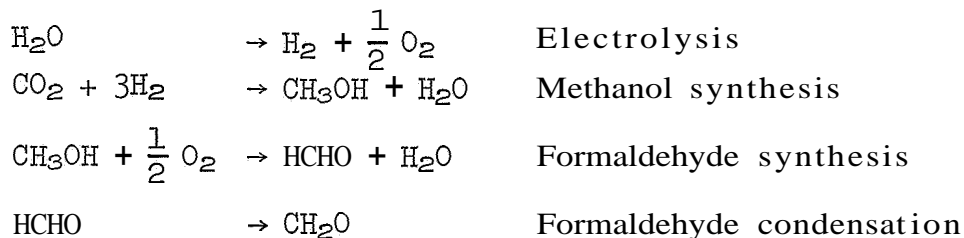


Figure 14. Carbohydrate synthesis, method 4.



The advantages of this method are (1) it eliminates two of the reaction steps required in method 2, methane formation (Sabatier reaction) and methane cracking or hydroforming; (2) each of its major reaction steps are exothermic; and (3) its processing steps require relatively low temperatures and pressures.

In the fifth method (fig. 15), concentrated CO_2 is reacted with hydrogen (water gas shift reaction) to obtain CO and water. The CO is separated from the water, more hydrogen is added, and the resultant mixture is sent to the

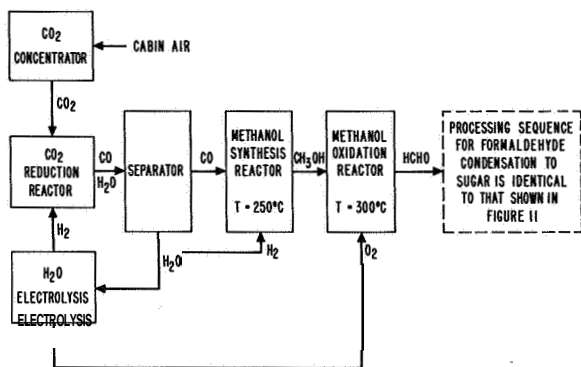
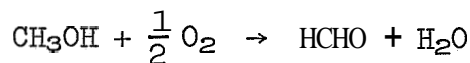
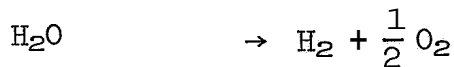


Figure 15. Carbohydrate synthesis, method 5.

methanol synthesizer. The remaining reactions leading to *sugar* production are similar to that of method 1. The reaction is shown below:



This method is advantageous in that CO is produced directly from CO₂, thus eliminating the methane cracking reaction (a high pressure, high temperature endothermic reaction).

In method 6 (fig. 16), CO₂ is reduced by H₂, producing HCHO directly. This has been accomplished experimentally by three different techniques:

(a) reduction of CO₂ by H₂ forming HCHO, and a reducing vapor was produced when the gases were finely divided by passing through a porous membrane with light used as a reaction promoter, (b) small quantities of HCHO were formed from a mixture of CO₂ and H₂ when the gas mixture was placed in a luminous discharge, and (c) HCHO and CH₃OH were synthesized from H₂ and CO₂ at 15 psi with a platinum gel catalyst. The formation of HCHO was favored.

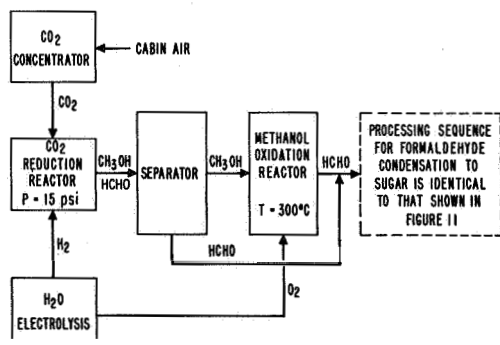


Figure 16.- Carbohydrate synthesis, method 6.

The formaldehyde synthesized in this manner is then processed to obtain sugars in a manner similar to method 1.

If high yields and efficiency can be obtained at reasonable energy expenditure for the direct conversion of CO₂ to formaldehyde, it would be highly advantageous from the standpoint of process simplification.

In the seventh method (fig. 17), the concentrated pure CO₂ is combined with hydrogen in a glow discharge reactor at low pressure (26 mm Hg) to form CO. This reaction is approximately 80 percent efficient and has an estimated yield of -110 g of CO per kWh. The unreacted CO₂ and hydrogen as well as the CO formed in the reaction proceeds to the silent discharge reactor. A portion of these gases is mixed with water before passing through the reactor.

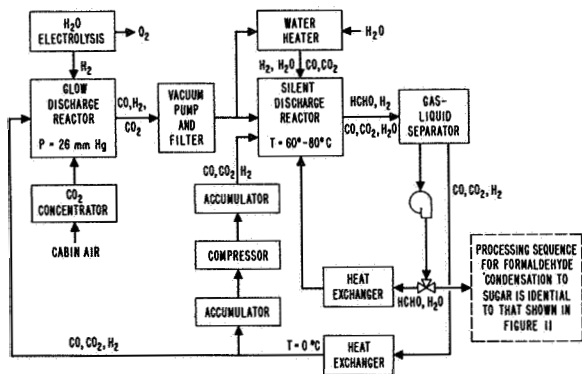


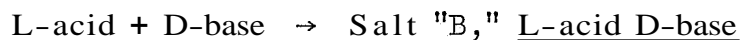
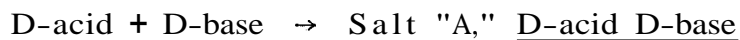
Figure 17.- Carbohydrate synthesis, method 7.

formaldehyde in the silent discharge reactor, ample water must be kept flowing through the reactor to dissolve the formaldehyde as it is formed and the temperature of the recycled mixture entering the reactor must be kept low. The formaldehyde reaction has an estimated yield of about 5 g HCOH/kWh and conversion efficiencies of as high as 70 percent have been suggested (ref. 73).

A portion of the formalin solution is recycled to the silent discharge reactor after being cooled to dissolve more formaldehyde. The remaining portion is processed in a manner identical with method 1 to obtain the sugar syrup.

Carbohydrate processing. - The carbohydrates synthesized by the preceding methods will contain many molecular structures. Only a portion of these sugars are known to be nutritional, and some may be toxic. Thus, separation of nutritional from nonnutritional sugars may be required.

Feasible methods for these separations are not evident. In some cases stereoisomers are separated by reacting with an optically active substance in the following manner:



Salt "A" and salt "B" will have different physical properties and are separable. No specific application of this technique to the separation of sugars is known.

The nonnutrient sugars may be oxidized to H₂O and CO or CO₂ as raw materials for recycling.

Advantages. -

1. System operation does not depend upon the viability of living organisms.

The reaction temperature is about 60° to 80° C. The formaldehyde formed in the silent discharge reactor is dissolved in the water passing through the reactor forming a formalin solution. The formalin solution and unreacted H₂, CO, and CO₂ then pass to a liquid-gas separator where the CO, CO₂, and H₂ are recycled to the silent discharge reactor for further formaldehyde production. In general, since the rate of formaldehyde formation is much lower than that of CO, the gas mixture passing through the silent discharge must be recycled several times. To decrease losses by decomposition of

2. The food yielded through physiochemical synthesis may be more readily controlled than that produced biologically.
3. Waste products of greatest mass (CO_2 and H_2O) from the crew's metabolism can be used in the production of the carbohydrates.
4. This method of producing carbohydrates is relatively insensitive to spacecraft environment.
5. Time required for carbohydrate production is less than by biological methods.

Disadvantages. -

1. The power requirement is relatively high.
2. The system is relatively complex.
3. The design of the system for zero gravity operation may be difficult.
4. Possible safety hazards may occur because of the high voltage or high temperature requirements of the system.
5. Heat rejection requirements are high.

Engineering data. -

1. Weight: Undetermined.
2. Method 7 requires 2.8 kW (ref. 70) to produce 690 g/day of hexose sugar. Scaled down to reflect production of 341 g/man-day of hexose sugar, which is approximately the normal amount of carbohydrate in man's diet, it is 1.38 kW/man.
3. Volume: Undetermined.

Development status and uncertainties. - The systems for physicochemical synthesis of carbohydrates are in the very early stages of development. Many investigators have studied the individual reactions that make up the overall carbohydrate synthesis reaction. Some work has been done on integrating these individual reactions to form a closed system for continuous carbohydrate production. All work, to date, has been on a laboratory scale with little emphasis on development of space-type hardware.

Uncertainties exist in the following areas:

1. The individual reactions do not appear to be integrated from the standpoint of balancing the available raw materials (CO_2 , H_2O , etc.) against the amount of carbohydrates that must be produced.

2. An improved reactor design and the discovery of more efficient catalysts could significantly reduce power requirements.
3. It has not been demonstrated that the carbohydrates synthesized are edible. Additional tests are required to determine the acceptability of the raw sugars in man's diet. It may be necessary to develop techniques for refinement of the raw sugars to remove any toxic components that might exist.
4. The synthesis processes must be adapted for use in a zero gravity environment.

Synthesis of Fats

Although man may be able to tolerate a diet devoid of fats, there is some evidence that certain fatty acids are essential. Thus, synthesis of fats as such may not be required, but synthesis of at least the essential fatty acids appears to be necessary for a completely closed ecology. Fats are esters formed by the reaction of organic acids with glycerol. Such esters are also classified as glycerides. Simple glycerides are those in which each glycerol molecule is esterified with three molecules of the same acid. Most fats and oils of a normal diet are mixtures of mixed glycerides. A mixed glyceride is one in which one molecule of glycerol has been esterified with two or three different acids. The esterification of fatty acids with glycerol to form fats is achieved simply by heating the reactants at suitable temperatures.

Synthesis of fatty acids. - An organic acid of a low molecular weight in the fatty acid series can be synthesized from the same intermediate compounds shown for carbohydrate synthesis as follows:



Fatty acids of higher molecular weights may be synthesized by starting with methane, which is another potential intermediate compound in carbohydrate synthesis.

The methane is converted to acetylene, which is subsequently converted to ethylene. Through recent technological developments on Ziegler-type catalysts, it is now possible to polymerize ethylene to terminal olefins of controlled chain length. Olefins having chain lengths in the fatty acid range (12-20 carbon atoms) are prepared commercially by this method. The conversion of the terminal olefins to fatty acids may be achieved by the "OXO" process or by oxidative cleavage of the ethylenic bond.

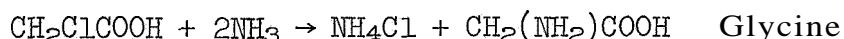
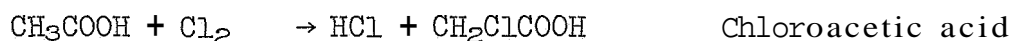
Synthesis of glycerol. - The common industrial method for synthesizing glycerol starts with fats, and if fats were available there would be no need to synthesize them. Another industrial method is to ferment sugar with yeast under special conditions. The use of a yeast culture is a departure from a pure physicochemical system and there is further the probability that a yeast

can be selected which will synthesize fats, thus eliminating the need for glycerol. A possible glycerol synthesis method starts with converting methane to acetylene. The acetylene is then reacted with formaldehyde to form propargyl alcohol, which is in turn hydrogenated to allyl alcohol and subsequently hydroxylated to glycerol.

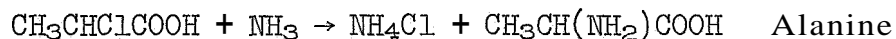
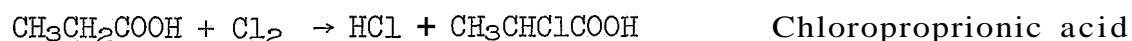
Development status and uncertainties. - There are no known developments in spacecraft hardware for synthesizing fats by physicochemical means. Some fragments of potential synthesis processes have industrial applications. Specific requirements for fats or fatty acids in the human diet have not been conclusively established. If it is determined that certain fatty acids are essential, it may be more economical to provide these in a stored supply, rather than by synthesis. The stored supply might be either a chemical dietary supplement or a conventional food.

Synthesis of Amino Acids

One method of synthesizing amino acids is by halogenating a fatty acid in the alpha position, as follows:



or



Only a few of the amino acids have structures with a direct counterpart in the fatty acid series. There may be other organic acids that react with halogens in a manner which leads to an amino acid.

Other methods for the synthesis of amino acids are described in following paragraphs.

In general, the yield of amino acids produced by these methods is quite low. The main objective of these synthesis experiments, however, has been to check theories concerning the formation of organic compounds under primitive earth conditions. As such, little attention was devoted to the efficient, high yield of edible amino acids.

Pansynthesis. - Pansynthesis (simultaneous synthesis of many) of amino acids has been achieved in the laboratory, but the yields have been extremely low. One method is to heat a mixture of "formose" with urea (ref. 74). In the best case, only 2 percent conversion of nitrogen in the reaction with urea was obtained. The reaction time was 10 days. There are also some unknowns as to nutritional value of the amino-acid mixture. Although 10 amino acids were identified in the mixture, these did not include all of those essential for man. There is also a nutritional preference for L-isomers. Certain D-isomers are known to substitute at least in part for corresponding L-isomers under certain conditions of diet. There is some

indication that polymers may be better accommodated by man than the free amino acids. Thermal panpolymerization, leading to proteinoids, has been demonstrated in the laboratory.

Synthesis by electrical discharge.- Neutral, basic, and acidic amino acids have been synthesized in milligram quantities by exposing a mixture of hydrogen, methane, water vapor, and ammonia to a high frequency spark discharge for a period of about one week (ref. 75). The gas mixture was maintained at a temperature of 70° to 80° C and a total pressure of 60 to 80 mm Hg. The products of this synthesis reaction were glycine, alanine, sarcosine, β -alanine, B₁, α -amino-butyric acid, and B₂.

Synthesis by irradiation.- Quantities of amines and amino acids have been synthesized by the action of ultraviolet light in the wavelength range of 1849 Å on a mixture of ethane, ammonia, and water (refs. 76, 77).

Considerable amounts of amino and fatty acids were synthesized by irradiation of ethane, ammonia, and water vapor with a mercury lamp for a period of six hours. The gas mixture was maintained at a temperature of 50° C and a total pressure of one atmosphere (ref. 78).

Amino acids have been synthesized by ultraviolet irradiation of a 2.5 percent solution of formaldehyde containing ammonium chloride or ammonium nitrate (ref. 79). A number of amines and amino acids have also been produced by irradiation of CH₄, CO₂, NH₃, N₂, H₂O, and H₂ by X-rays (ref. 79).

Catalytic synthesis. - It has been shown experimentally that serine and threonine peptides can be synthesized through the catalytic surface reaction of formaldehyde and acetaldehyde with polyglycine adsorbed on kaolinite.

Development status and uncertainties. - There are no known developments in spacecraft hardware for the synthesis of proteins or protein derivatives of physicochemical means. Some fragments of potential synthesis processes have been demonstrated in the laboratory. All of the essential amino acids have been synthesized by industrial methods, but these have not been limited in raw materials to man's metabolic wastes. Some of the amino acids are required in such small quantities that a stored supply may be more economical than means for synthesis. The stored supply might be either in a chemical dietary supplement or in conventional food.

Physicochemical Waste Processing

The principal materials for food synthesis are CO₂ and H₂ which may be obtained by processes such as water electrolysis and concentration of metabolic CO₂ from the spacecraft atmosphere. Additional small amounts of CO₂ and H₂O may be produced by oxidation of organic material in the solids of urine and feces. Several processes for achieving this oxidation, as well as providing for the reduction of waste solids and water recovery, are discussed below. This is not intended to represent mention of all candidate processes.

Zimmerman wet oxidation process. - The basic principle involved in the wet oxidation of wastes is the combustion of these wastes with air at high pressures, oxidizing organic matter dissolved or suspended in water. The wastes are burned in the presence of water as completely as if the water were evaporated before combustion. The end products in both cases are steam, nitrogen, CO₂, and ash (refs. 80-82).

Process description. - As shown in figure 18, the waste products generated onboard the spacecraft are wet-ground in the waste pulverizer and blender to form a homogeneous slurry. The waste slurry is then passed through a preheater where it is heated by hot effluent returning from the oxidation reactor. The waste is pressurized to approximately 1800 psig and mixed with compressed air at the same pressure. It passes through a series of regenerative heat exchangers where heat is picked up from the hot oxidized effluent returning from the reactor, and the waste slurry is raised to the required temperature. The heat required for starting the process is provided from an external source.

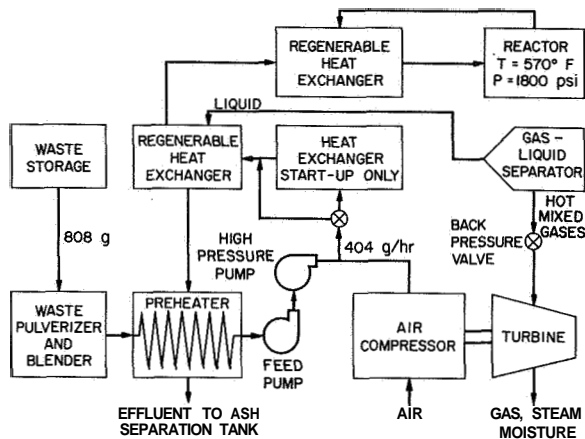


Figure 18.- Schematic diagram of the Zimmerman wet oxidation waste reduction system.

The waste slurry and air mixture enters the reactor and is oxidized, liberating additional heat.

After leaving the reactor, the liquid and gas of the effluent are separated. The liquid passes back through the regenerative heat exchangers, giving up its excess heat to incoming waste slurry. The hot, high-pressure gases are expanded through a turbine and thus cooled. The turbine provides the power to drive the air compressor. A back pressure valve continuously discharges the water effluent. Since the oxidation process proceeds smoothly only in the presence of liquid water, care must be taken to insure that ample water is provided in the reactor throughout waste oxidation.

Advantages :

1. Dehydration of wastes is not required prior to oxidation.
2. Little excess air is required for combustion.
3. All solid organic matter is effectively removed from the effluent except for insoluble ash, leaving an effluent similar to that from an anaerobic digestion process.
4. Wastes are processed rapidly.

5. The power requirement is low because energy is recovered from the exothermic reaction.
6. The volume requirements are low.
7. Reduction depends only upon a chemical reaction rather than on the viability of a biological system.
8. The process is relatively easy to operate and maintain.

Disadvantages :

1. The reactor would be difficult to operate with a two-phase fluid under zero gravity.
2. The high pressure and temperature of the fluid presents a possible explosion and burn hazard.
3. The product of the process still needs further processing before potable water is obtained.
4. Oxygen is required for waste reduction.

Engineering data: The estimated weight and power required for a Zimmerman process unit to handle the wastes of a 10-man crew are 40 kg and 130 W.

Development status and uncertainties: This process has not been developed for space use although a number of commercial plants use this system to process city sewage sludge. Uncertainties connected with the use of this system include:

1. The plants built to date are more than 1000 times larger than needed for space use. The operating Characteristics of such a unit when scaled down by a factor of 1000 must be determined.
2. The composition of the effluent required for use in food generation is not established.
3. The composition of the waste to be processed by this method cannot be established until a diet is defined.

Waste reduction and water recovery by a combustion process. -

Process description: A schematic diagram of the system is shown in figure 19 (ref. 83). Urine and feces are supplied to the system along with air. Air and the wet wastes are compressed separately and passed through a regenerative heat exchanger to a combustor. All water is vaporized in the heat exchanger. Solid organic material enters the combustor and is burned at approximately 1000° F. The stream of combustion products reenters the

regenerative heat exchanger where a portion of heat of combustion is removed, partially condensing the water vapor contained in the stream. When the stream temperature is about 700° F, the stream

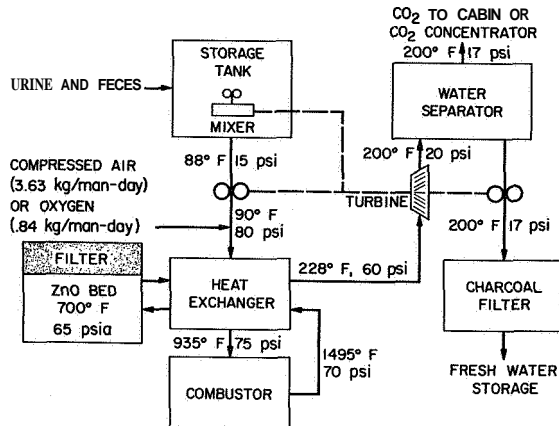


Figure 19.- Schematic diagram of the waste combustion and water recovery system (ref. 86).

temperature is about 700° F, the stream is routed from the heat exchanger through a zinc-oxide bed and a solids filter which remove sulfur dioxide and solid particles. The stream then reenters the heat exchanger and is cooled further. After leaving the heat exchanger, the stream is expanded through a turbine to recover useful energy and for further cooling. The turbine drives the storage tank mixer and the fluid pumps. The gas stream passes through the water separator, where entrained water is removed, and then to the cabin. The water removed by the separator is passed through a charcoal filter to remove dissolved components and then is stored.

Advantages :

1. Low power requirement.
2. Low fixed weight requirement.
3. Oxidizes harmful organic matter and bacteria.
4. High integration potential.
5. Provides for fecal water recovery.
6. High water recovery efficiency.

Disadvantages :

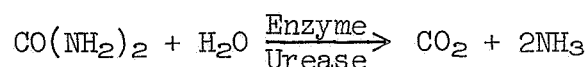
1. High expendable weight (oxygen and charcoal).
2. Relatively complex.
3. Possible safety hazard (burn and contamination).
4. Based on continuous process, when operation may have to be cyclic.

Engineering data:

1. Weight: Unknown.
2. Power: 15 W/man (ref. 83).
3. Volume: Unknown.

Development status and uncertainties: This system is in a conceptual stage and needs experimentation to determine the validity of theoretical heat transfer rates, power requirements, and process rates. It must also be determined whether the waste residue produced by this process is useful.

Waste processing for nitrogen recovery. - Nitrogen is required for synthesis of amino acids. Most of the nitrogen in wastes is as urea, and most of the mea is in the urine. The processing to recover nitrogen will depend on the process for synthesizing the amino acids. Use of the nitrogen as urea would require separation from urine. Use as ammonia may involve hydrolysis of the urea as follows:



The processing would probably be less complex if the above reaction were accomplished in the urine, followed by separation of gaseous ammonia from the liquid residue.

Incineration. - Products from incineration may be useful in biosystems. Present procedures, however, do not permit quantitative conversion of feces and urine to nutrients useful in biosystems. The carbon in the waste is not quantitatively converted to CO_2 , the ash generated is not totally soluble, and gaseous nitrogenous products require further processing for conversion to products suitable for biosystems. A loss of at least 0.2 percent of the CO to the ash and an ash solubility of 75 to 85 percent is estimated (refs. 17 and 21).

Other waste-processing methods. - Information similar to the foregoing is available from previous programs on other subsystems for solid waste reduction and water recovery. The water recovery subsystems considered were electro dialysis adsorption, vacuum distillation-pyrolysis, vapor compression-adsorption, air-evaporation-absorption, vacuum distillation-adsorption, and multifiltration. Less detailed information is available on such water recovery techniques as reiterative freezing, freeze crystallization, freeze sublimation, ultrafiltration, reverse osmosis, osmionic process, thermo-osmosis, electrolysis, multieffect evaporation, thermoelectric distillation, solvent extraction, electrochemical treatment filtration, ion exchange, and hydrate formation (ref. 84). The solid waste-processing techniques considered were freeze-drying, thermal decomposition, and incineration (ref. 85).

Other Subsystems

Whether food is synthesized and waste is reduced biologically or physico-chemically, certain other subsystems are needed to fulfill such functions as separating CO_2 from the cabin air, producing and storing oxygen, electrolyzing water, and controlling contamination. Table V indicates some of these subsystems that may have application to a closed ecology.

TABLE V. - SUBSYSTEMS FOR REGENERATIVE LIFE SUPPORT SYSTEMS

Function	Subsystem or technique
CO ₂ removal and concentration (ref. 86)	Molecular sieve adsorption Solid amine adsorption Freeze-out Electrodialysis Diffusion through membranes
CO ₂ reduction (ref. 86)	Bosch reaction subsystem Sabatier reaction subsystem Solid electrolyte subsystem Molten electrolyte subsystem Thermal dissociation at reduced pressure
Water electrolysis (ref. 86)	Double membrane cell Alkaline electrolyte cell Hydrated phosphorus pentoxide cell Rotating electrolysis unit Palladium cathode-hydrogen diffusion unit Solid-state electrolytic cell
Contaminant control (ref. 87)	Activated charcoal Catalytic burner - palladium catalyst Catalytic burner - hopcalite catalyst Catalytic oxidation unit

TECHNICAL DISCUSSION, CLOSED FOOD-WASTE LOOP

The Nutrient Balancing Problem in a Closed Ecology

A critical area in closing the ecology aboard a spacecraft is balancing nutritional component synthesis with dietary requirements of the crew. The culture of Hydrogenomonas is an example. A favorable characteristic is that it can utilize man's waste CO₂ and urea to grow bacterial cells which are potential food for man. Quantities of wastes per man-day include about 35 g of urea and 1000 g of CO₂. Unfortunately, the bacteria normally do not use the wastes in these proportions. If all the urea is used, only about 20 percent of the CO₂ will be taken up. Another indicator of the imbalance is the protein fraction of Hydrogenomonas relative to the protein fraction of a normal diet. The Hydrogenomonas cells contain over 50 percent protein (refs. 88 and 89), while recommended diets usually show protein in the 10 to 20 percent range. The minimum dietary requirement may be as low as 7 percent (35 g protein in 500 g of dry food). If all the nitrogen in man's waste is converted to protein by the bacteria, if the bacterial cells are converted to food without protein loss, and if the bacterial cells contain at least

minimum dietary requirements of the essential amino acids, then, the Hydrogenomonas culture may be capable of supplying the total protein requirement. However, it will have utilized only about 20 percent of the waste carbon and there will be a diet deficiency in carbon as carbohydrates or fats. The lack of balance involves primarily nitrogen and carbon. There is little nitrogen in man's wastes other than in urea and little carbon in man's wastes other than in CO₂.

Cultures of algae or other microorganisms have a similar problem in the carbon/nitrogen ratio. Their cells, too, normally have a protein content of more than 50 percent. There are some possibilities that microorganisms can be developed to yield acceptably low protein products. It has been reported that algae may vary in protein content. Hydrogenomonas were grown under conditions of nitrogen deficiency from cell material rich in carbohydrate and lipids. The lowest nitrogen content observed under these conditions was 4 percent (ref. 88). There was also a decline in biosynthetic rate as indicated by a decline in gas uptake. There is insufficient experimental evidence to conclude that these approaches are feasible.

Chemical synthesis of one or more dietary components may be necessary for achieving a satisfactory elemental balance in the food-waste loop.

Closed Configurations

It is evident that food production is the key process in a complete food-waste loop and that processing the waste is necessarily closely integrated with food production. Once the closed food-waste loop is established, other subsystems can be selected to complete the closed ecological system.

The closed configurations may be categorized as: (1) biological, in which biological subsystems are used for all food production and for processing all solid wastes; (2) physicochemical, in which there are no biological components other than man himself; and (3) composite, in which both biological and physicochemical subsystems are elements of the food-waste loop.

Biological subsystems food-waste cycle.- A hypothetical configuration for using biological subsystems to close the food-waste loop is shown in figure 20. The subsystems are an activated sludge waste processing unit and a Hydrogenomonas culture for food production. Feasibility of this configuration rests on many assumptions, the most critical being that of a closed nitrogen loop. This is discussed further in a subsequent paragraph.

Process description: Hydrogenomonas are grown in a continuous culture apparatus at a density of 10 g/liter in a culture volume of 100 liters/man. A continuous cell harvest is accomplished by separating suspended solids from a recirculating flow of the culture medium. The harvested cells are processed for food, and the food product is stored for only the brief periods necessary to match production and consumption. The liquid remaining after cell separation is divided so that part returns directly to the culture vessel and the remainder is processed to remove contaminants excreted by the bacteria. The first processing step is water recovery, in which water is

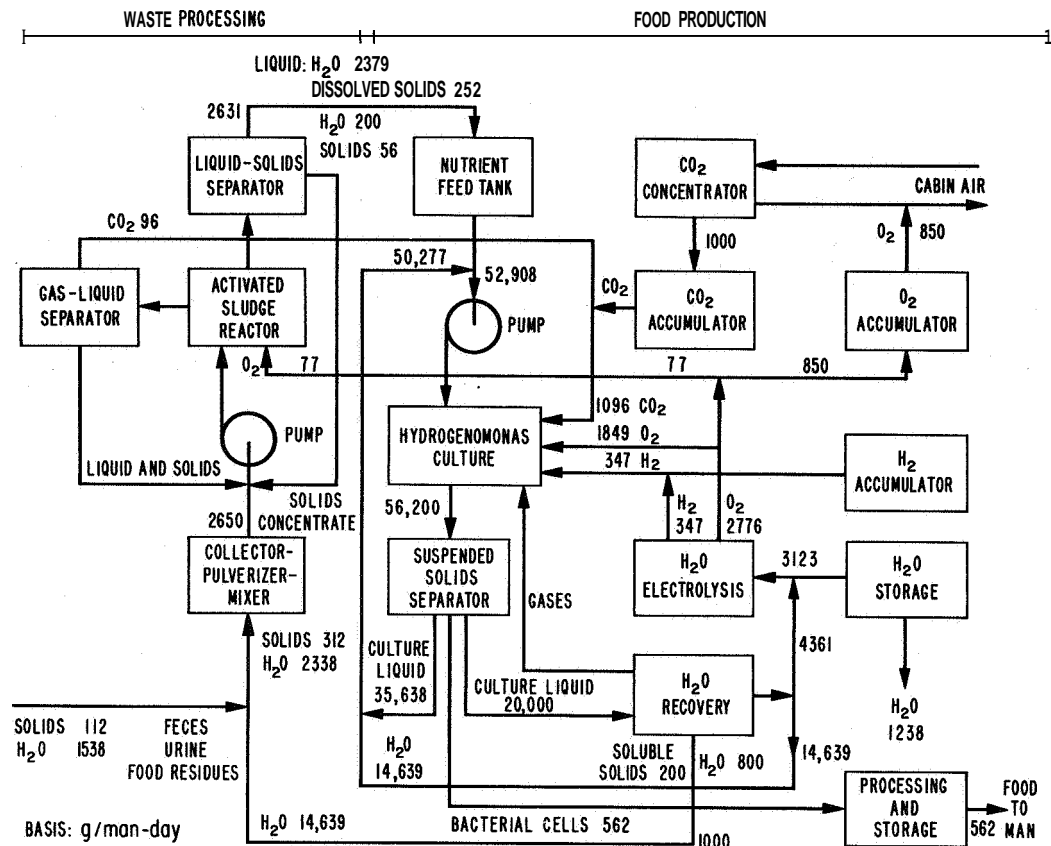
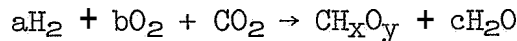


Figure 20.- Biological subsystems food-waste cycle flow balance.

separated from dissolved solids. Part of the water is returned to the bacterial culture to maintain the material balance. The excess water formed in the bacterial reaction is stored for subsequent electrolysis. This excess results from the reaction:



Soluble solids from the water recovery unit are transferred to the waste processing unit for conversion to nutrients for the bacterial culture.

Gases taken up in the bacterial reactor are provided at controlled rates so that concentrations in the culture medium are satisfactory for cell growth and reproduction. Hydrogen and oxygen are provided from the water electrolysis unit, which also provides oxygen to the cabin atmosphere for consumption by the crew. Carbon dioxide exhaled by the crew is separated from the cabin atmosphere by a CO₂ concentrator and then transferred to the bacterial reactor. The gas supply apparatus includes accumulators for each gas to accommodate surges in production and consumption.

Other nutrients for the bacteria are provided as a liquid concentrate from a feed tank that discharges a metered flow into the recirculating culture liquid. This dilution of the concentrate assists in mixing nutrients into the cell suspension. The concentrate is the liquid effluent from the waste processing unit. Inputs to the waste processing unit include urine, feces, and the residue from water recovery units.

Solid wastes are suspended in sufficient liquid to permit transfer by conventional pumping techniques. The wastes are mixed, pulverized, and pumped into the activated sludge reactor for further bacterial action. The liquid effluent is delivered to the nutrient feed tank of the Hydrogenomonas culture.

Assumptions: The assumption of a closed nitrogen loop may be construed as: (1) a nitrogen-deficient culture for the Hydrogenomonas, whereby the bacteria adapt to the substrate and metabolize cells of abnormally low protein content (this protein level, however, is in the normal range for the human diet); or (2) a nitrogen-sufficient culture for the Hydrogenomonas, resulting in normal protein metabolism by the bacteria and an abnormally high protein intake by the crew (it must then be assumed that the crew excretes wastes of the high nitrogen content required by the bacteria); or (3) an adjustment to an intermediate level such that the bacteria receive less than optimum nitrogen and the crew diet contains more than the normal protein content.

Some other assumptions inherent, in the configuration shown are:

1. Harvested cells are nutritionally adequate for man, except for minor supplements of minerals and vitamins.
2. Harvested cells are readily digested by man or can be readily processed to high digestibility.
3. Harvested cells can be processed to a palatable flavor, or to a flavorless condition that will permit adding a variety of artificial flavorings.
4. The waste processing system will recover nutrients, especially nitrogen compounds, in a form the Hydrogenomonas can utilize. Other nutrients are necessary and are expected to be available from wastes, but the quantities are relatively small and a stored supply is probably feasible.
5. Metabolic waste products of the bacteria will be degraded to nutrient materials in the activated sludge reactor.

Configuration alternates: A number of modifications and alternate configurations are conceivable. Some may have application as follows:

1. Any N_2 contamination of the gases fed into the Hydrogenomonas culture would presumably cause an accumulation there. There may be a requirement to provide a purge flow from the gases returned to the culture from the water recovery unit. This purge would be through a

catalytic burner with discharge to the cabin air. Thus, the N₂ that entered the loop from cabin air would be returned to the cabin air.

2. A purge to remove inorganic salts from the Hydrogenomonas culture liquid may be required.
3. H₂ and O₂ can be provided by direct electrolysis of waste liquids, rather than by processing to pure water first.
4. Culture liquid processed through the water recovery unit creates more potable water than is required for electrolysis. Instead of this water returning directly to the Hydrogenomonas reactor as shown in figure 20, it may be integrated with the entire water of the life support system. This water could be used for washing the Hydrogenomonas cells, as part of the food processing operation, without detriment to subsequent return to the culture vessel.
5. There are several applicable types of CO₂ concentrators and H₂O recovery units.

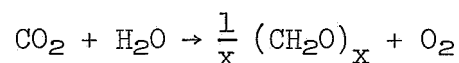
Physicochemical subsystems food-waste cycle. - The present level of technology does not provide a basis for establishing a complete closed food-waste cycle with only physicochemical subsystems. Waste-processing subsystems which may be applicable and potential processes for producing some food components have been discussed in preceding paragraphs.

Composite food-waste cycle. - The combining of biological with physicochemical subsystems permits exploiting the particular advantages of each type. Food produced from normally cultured microorganisms is too high in protein to be used by itself as the sole nutrient for man.

Physicochemical synthesis of carbohydrates appears to have more promise than synthesis of proteins or protein derivatives. Thus, the best combination of biological with physicochemical subsystems in a food-waste cycle will utilize the protein-producing capabilities of the biological subsystem along with physicochemical synthesis of carbohydrates.

Process description: Closed-loop conversion of wastes to food is performed by three major subsystems. An activated sludge waste-processing unit converts urine and solid wastes to nutrients for a Hydrogenomonas culture. The Hydrogenomonas cells are harvested and processed to food for the crew, thereby providing their total protein intake. A carbohydrate synthesis unit uses raw materials of CO₂ and H₂ to make a mixture of sugars. These carbohydrates are a necessary supplement to the Hydrogenomonas food product to achieve a satisfactory carbohydrate/protein ratio in the diet.

The carbohydrate is synthesized by a series of catalytic reactions which has the net result'



The term $(\text{CH}_2\text{O})_x$ represents a mixture of sugars. This mixture will probably include a number of hexoses and pentoses. It will also have both dextro and laevo stereoisomers. If it is determined that some of these are toxic, a separating process is required and only some fraction of the sugars synthesized will be used as food for man. The remaining sugars are oxidized and cycled back through the synthesis process.

Assumptions: The feasibility of this composite food-waste system depends on the validity of several major assumptions as follows:

1. A nutrient balance can be achieved. The Hydrogenomonas culture is the only means of recovering nitrogen in man's wastes and returning it to his diet. The elements carbon, hydrogen, and oxygen are consumed in different ratios by the Hydrogenomonas culture and the sugar synthesis unit. This may permit the adjustment of synthesis rates to achieve a balance for these elements. There are other essential elements, such as calcium, phosphorus, iron, sulfur, etc., which may be inadequate for the Hydrogenomonas from man's wastes, or for man from the Hydrogenomonas cells. Nutritional supplements for these elements would probably involve relatively small weight penalties. However, the use of such supplements would mean that an equal mass in wastes would be unusable. There may be a problem in separating such constituents of the wastes.
2. All solids are degraded to either gases or soluble materials in the activated sludge reactor. A recycle loop is provided so that insoluble solids are returned to the reactor for further action by aerobic bacteria. If equilibrium conditions involve an excessive return of undegraded solids, it may be necessary to discard such refractory materials. An alternate would be to provide other means of degradation, such as by sonification or oxidizing in an incinerator. Complete conversion of wastes to raw materials for food synthesis is essential for closing the food-waste loop.
3. The nutrient and the nonnutrient components of the synthetic sugar mixture can be separated by physicochemical techniques. Processes specifically applicable to such a mixture are not known.
4. A high mass fraction of the sugars of the mixture are satisfactory as nutrients for man. A low fraction would reduce process efficiency, possibly beyond the limits of practicability.
5. The harvested Hydrogenomonas cells and the synthetic sugars are nutritionally satisfactory and can be processed to acceptable flavors for use as food. Minor supplements of minerals and vitamins may be required, and are considered to be within the concept of a closed food-waste cycle. However, the Hydrogenomonas food product should supply substantially the total amino acid requirement.

ENGINEERING EVALUATION OF CLOSED CONFIGURATIONS

This section documents engineering evaluations of closed food-waste configurations for space applications consistent with the System Model. Weight, power, and volume estimates are made. Equivalent weight penalties for electrical power, process heat, and heat rejection are in accordance with the System Model. A comparison is made with a stored food system, which also is based on the 10-man crew and 6000 man-days requirement of the System Model. From prior studies, the 6000 man-day weight and volume baselines are established at 5500 kg and 34,000 liters for food, packaging, storage, and preparation facilities. No credit is allowed for water as stored food since it will become a surplus in a closed loop. The adjustments in weight and volume for differences in total function are based on studies under Contract NAS 1-2934 (ref. 1) and the amounts are shown in table VI. The stored food system baseline is used primarily in the context of evaluating closed food-waste configurations relative to one another. Evaluations in greater depth, for a specific mission, are recommended before conclusions are drawn from a comparison of closed vs. open systems.

The following data are for adjusting weight and volume estimates of closed food-waste configurations in comparison with a stored food system.

TABLE VI. - DATA FOR ADJUSTING WEIGHT AND VOLUME ESTIMATES

<u>Per man for a 10-man system</u>	<u>Weight, kg</u>	<u>Volume, liters</u>
Oxygen recovery	117	28
Urine water recovery	14	85
Feces drying and storage	20	21

Notes :

1. Based on flight version estimates for the NASA/Langley four-man system, Contract NAS 1-2934 (ref. 1).
2. Penalties for electrical power, process heat, and heat rejection are included, based on the system model for this program.
3. Oxygen recovery includes CO₂ separation from cabin air, CO₂ reduction, and H₂O recovery.
4. Urine water recovery data are for recovery only. Collection, transfers, and storage are not included.
5. Feces storage data have been adjusted to the concept of dual use of the waste containers. They would be used initially for storage of food.

Engineering estimates were made only for those configurations which were judged to be competitive for the conditions of the System Model. Qualitative evaluations only are given for configurations that were categorized as noncompetitive.

Engineering Evaluation of Biological Subsystems

Three biosystem configurations incorporating Hydrogenomonas were evaluated. The three systems were termed: (1) Hydrogenomonas-Activated Sludge, Solids Purge; (2) Hydrogenomonas-Activated Sludge, Ultrasonic Loop; and (3) Hydrogenomonas-Activated Sludge, Anaerobic Loop; respectively. The configurations were selected to compare the effect of a solids purge and maximum biological transformation of waste solids possible on overall system rating. Except for the disposition of suspended organic matter from the activated sludge reactor, the three configurations are essentially the same.

Hydrogenomonas-Activated Sludge, Solids Purge. - A schematic of the Hydrogenomonas-Activated Sludge, Solids Purge configuration is given in figure 21. The Hydrogenomonas subsystem is utilized for food production and the activated sludge subsystem for waste treatment.

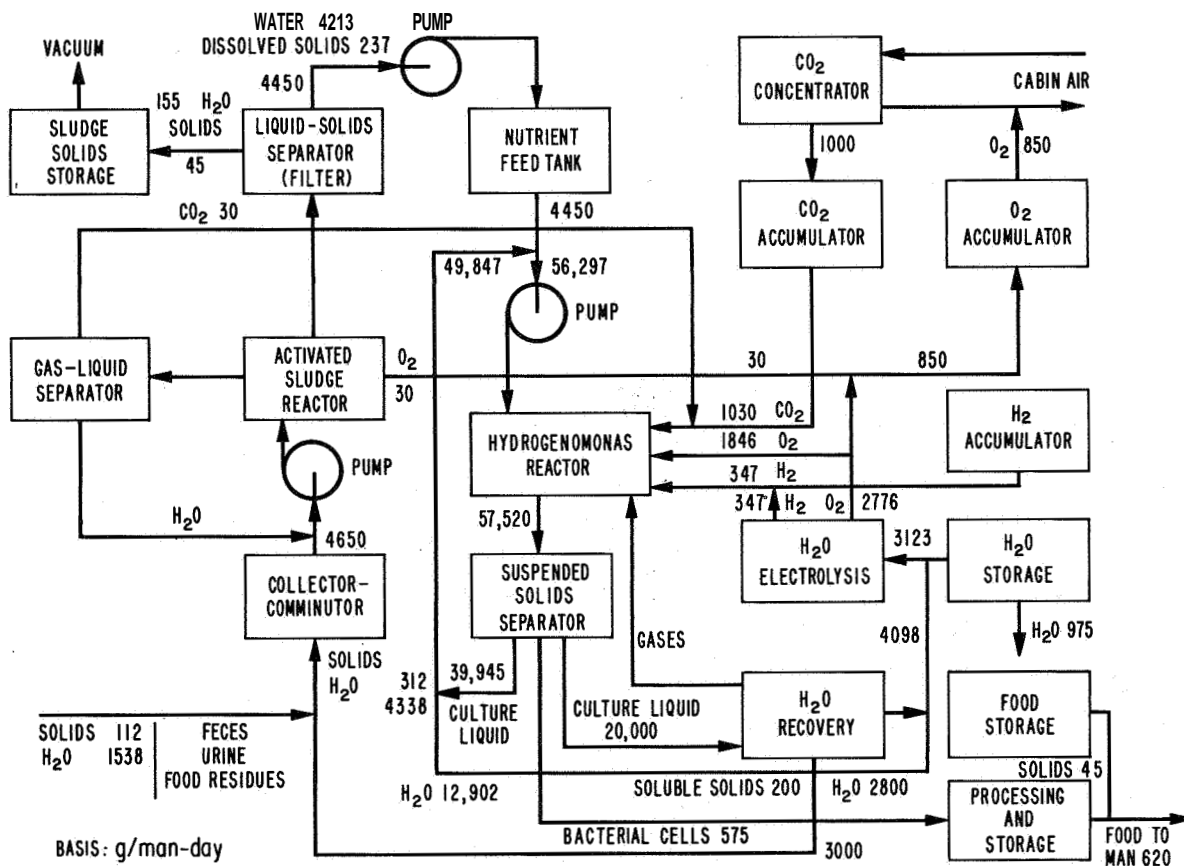
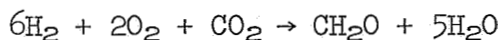
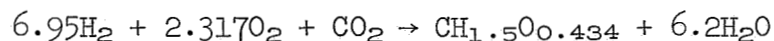


Figure 21.- Hydrogenomonas-activated sludge, solids purge.

Process description: The Hydrogenomonas culture has been described in preceding paragraphs. Process flows shown in figure 21 are calculated from data of the system model and from the stated characteristics of the Hydrogenomonas culture. The Hydrogenomonas biosynthesis has been represented in the literature as:



However, the experimental results reported have shown a very wide range in mole ratios. In the absence of consistent data, an arbitrary approach was adopted which is based on the observation that the mass fraction of CO_2 taken up is approximately one-third of the total gas uptake. This permits calculation of an empirical reaction as follows:



It is assumed that 20 percent of the culture medium (100 liters/man) will be withdrawn daily for processing to remove contaminants. This imposes an additional process load of 20,000 g/man-day on the water recovery apparatus. Also, to harvest 575 g/day from a suspension of 10 g of cells per liter will require a flow into the separator of 57,500 g/day.

The foregoing accounts for the principal elements involved and is the basis for flows shown. Other nutrients for Hydrogenomonas, although very essential, involve relatively small masses and will have a negligible effect on flows required. Corollary to the concept of additional nutrients is that of a corresponding mass in wastes, particularly inorganic salts, which the bacteria cannot use. This may require a small purge flow, either continuous or intermittent, of culture liquid concentrate into a water recovery unit. For example, NaCl, would thus be removed to avoid a detrimental concentration in the Hydrogenomonas culture. The salts might be of a quality suitable for man to use in his food.

Assumptions: The principal assumptions for the Hydrogenomonas culture are as previously stated. The most important is that man can process and consume the bacterial biomass generated to achieve a nitrogen balance or Hydrogenomonas will grow in a nitrogen-deficient culture. Assumptions for the activated sludge unit are:

1. Wastes at high waste solids concentrations can be processed by the activated sludge process.
2. Microbes can be grown at culture densities of 10 g (dry weight)/liter.
3. The stoichiometric gaseous relationships of water electrolysis, hydrogen bacteria, activated sludge, and man will balance.

Engineering estimates: Estimates of weight, volume, process heat, electrical power, and heat rejection are summarized in table VII. The mass of undegraded organic material transferred to waste storage from the activated sludge subsystem is estimated to be 45 g/man-day. The weight penalties,

TABLE VII. - VOLUME-WEIGHT BREAKDOWN OF THE HYDROGENOMONAS-ACTIVATED SLUDGE, SOLIDS PURGE CONFIGURATION

Item no.	Description	Volume, liters	One man, 600 days; weight, ~ kg				Process heat, W	Heat rejection, W	Process heat, W				
			Fixed	Expendables	Power maintenance	Heat maintenance				Process heat	Total penalty		
1	O ₂ accumulator	40	6										
2	N ₂ accumulator	140	21								21		
3	N ₂ O storage	40	50								50		
4	Food storage	200	1	27							28		
5	Food processing	100	20	7	0.5	0.4	0.2	10	25	15	27.9		
6	CO ₂ concentrator	100	16		4.5	12.0	10.7	90	800	710	32.5		
7	CO ₂ accumulator	20	3		41	12.3		820	820		3		
8	H ₂ O electrolysis	220	55		3.4	11.5	10.5	67	767	700	108.3		
9	H ₂ O recovery	355	45		0.1			2	2		15.0		
10	Nutrient feed tank	10	15		0.1			2	2		0.1		
11	Nutrient feed pump				15.0	4.5		300	300		139.5		
12	Hydrogenomonas reactor	120	120		0.5	0.4		10	10		1.9		
13	Suspended solids separator	1	1								1.0		
14	Liquid-solids separator	1	1								11.7		
15	Activated sludge reactor	10	10		1.3	0.4		25	25		0.1		
16	Activated sludge feed pump				0.1			2	2		0.1		
17	Activated sludge gas-liquid separator	1	1		0.3	0.1		5	5		1.4		
18	Waste collector-pulverizer	280	40		0.7	0.2		13	13		40.9		
19	Sludge storage	150	2								2.0		
20	Activated sludge effluent pump				0.1			2	2		0.1		
	Subtotal	1788	407	34	67.5	41.8	21.4	1346	2771	1425	550.3		
	20 percent plumbing instrumentation	358	81.4		13.5	8.4	4.3	269	554	285	103.3		
	Total	2146	488.4	34	81	50.2	25.7	1615	3325	1710	679		
	Adjustments	-134									-151		
	Adjusted total	2012									528		

based on the system model, are 15 kg/kW for heat rejection and process heat and 50 kg/kW for electrical power. The total weight penalty is the sum of the individual penalties named plus the component hardware weight and the weight of stored food to compensate for undegraded wastes transferred to storage.

Hydrogenomonas-activated sludge sonification. -

Process description: This configuration (fig. 22) is similar to the Hydrogenomonas-activated sludge, solids purge configuration except that an

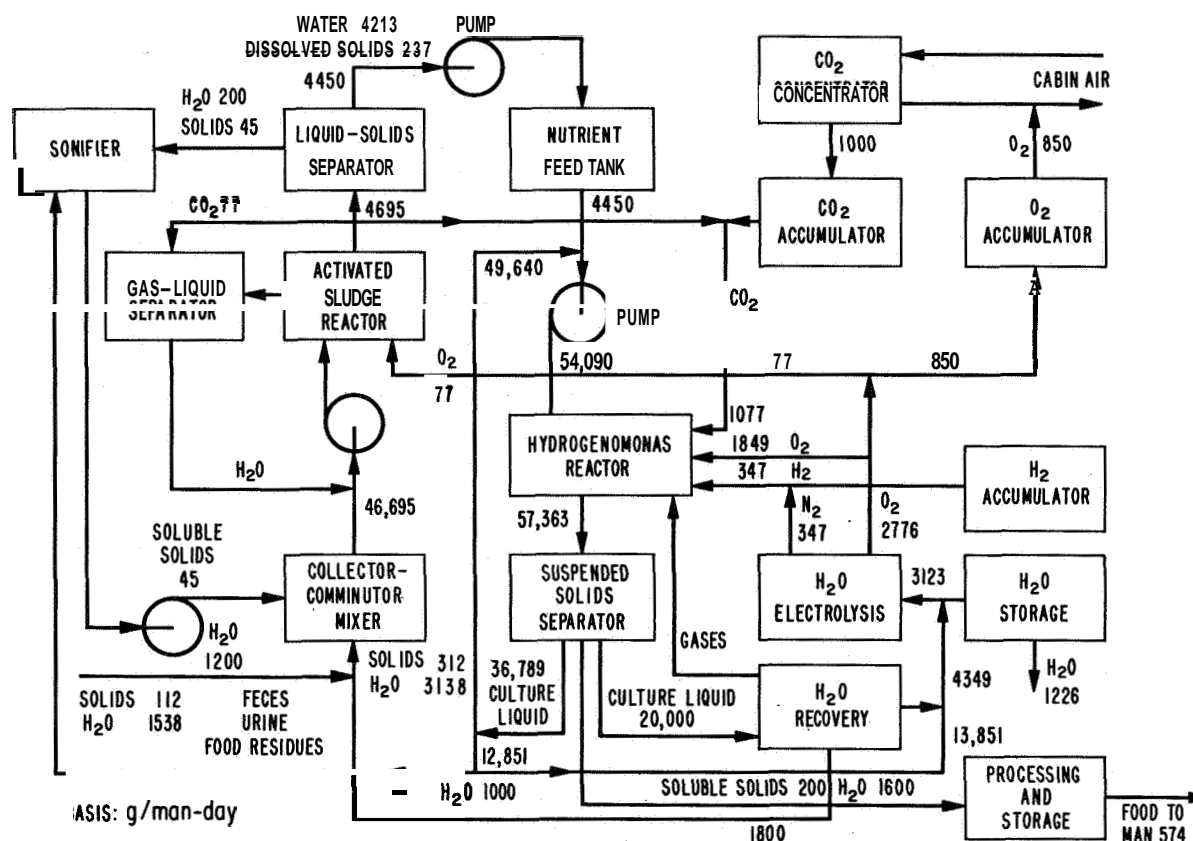


Figure 22.- Hydrogenomonas-activated sludge, sonification.

additional processing step has been added. Disruption of microbial cells by ultrasonic energy is employed to obtain maximum transformation of wastes to nutrients for the Hydrogenomonas subsystem. This processing step is termed "sonification." Alternate procedures of cell rupture or direct utilization of the activated sludge biomass generated are not excluded. The major advantage of such a system is that maximum recycling of matter is achieved. Feasibility of the configuration is contingent on the success of the processing step and/or composition of wastes that will have to be processed.

Assumptions: In addition to the assumptions listed earlier, complete degradation or utilization of suspended matter in the waste treatment process must be assumed.

TABLE VIII. - VOLUME-WEIGHT BREAKDOWN OF THE HYDROGENOMONAS-ACTIVATED SLUDGE, SONIFICATION CONFIGURATION

Item no.	Description	Volume, liters	One year, 000 days; weight, ~kg				Process heat, °C	Heat rejection, °C	Power, W	Process heat, °C
			Fixed	Expendables	Power	Heat				
1	O ₂ accumulator	40	6							
2	H ₂ accumulator	140	21							
3	H ₂ O storage	40	50							
4	Food processing	100	20	7	0.5	0.4	25	10	15	
5	CO ₂ concentrator	100	16		4.5	2.0	800	90	710	
6	CO ₂ accumulator	20	3							
7	H ₂ O electrolysis	220	55		41	2.3	820	820		
8	H ₂ O recovery	355	45		3.4	1.5	767	67	700	
9	Nutrient feed tank	10	15							
10	Hydrogenomonas reactor	120	120		15.0	4.5	300	300		
11	Suspended solids separator	1	1		0.5	0.4	10	10		
12	Activated sludge effluent pump									
13	Liquid-solids separator	1	1		0.3	0.1	2	2		
14	Activated sludge feed pump				0.1		5	5		
15	Waste collector-pulverizer	280	40		0.7	0.2	2	2		
16	Sonifier	1	1		0.4	0.1	13	7		
17	Gas-liquid separator	1	1		0.3	0.1	5	5		
18	Sonifier effluent pump				0.1		2	2		
19	Nutrient feed pump				0.1		2	2		
20	Activated sludge reactor	10	10		1.3	0.4	25	25		
	Subtotal	1439	405	7	68.3	42.0	2785	1360	1425	
	20 percent plumbing instrumentation	287.8	81		13.7	8.4	557	272	285	
	Total	1727	486	7	82	50	3342	1632	1710	
	Adjustments	-134								
	Adjusted total	1593								

Assumptions: In addition to the assumptions listed under earlier paragraphs, the availability of degradation products of the anaerobic process to the Hydrogenomonas subsystem must be assumed.

Engineering data and rating: Weight and volume estimates of components in this configuration are given in table IX.

Engineering Evaluation of Physicochemical Subsystems

Currently, the most promising physicochemical technique for food-loop closure is the synthesis of carbohydrates from man's metabolic waste products. In order to make a valid comparison of carbohydrate synthesis with stored food or other methods of food production, this technique must be charged with a penalty reflecting the addition of another subsystem to supply the protein required in man's diet. Physicochemical synthesis of protein or its derivatives is judged at present to be noncompetitive. Consequently, for the purpose of evaluation, a penalty corresponding to a stored protein derivative supply was added to the carbohydrate synthesis subsystems.

The weight and volume of this stored protein derivative supply were calculated using the following assumptions:

1. The weight corresponds to data for amino acids given in table X (based on ref. 90).
2. The weight of the packaging is equal to 12 percent of the weight of the stored protein derivatives.
3. The density of the protein derivatives is similar to that given for amino acids in reference 91.

Carbohydrate synthesis systems. Several methods for synthesis of carbohydrates or "sugars" from man's metabolic waste products are described in preceding sections. Evaluations were made for two that have sufficiently defined characteristics to permit engineering estimates.

The first method is shown schematically in figure 24. The basic raw materials of CO_2 and hydrogen are converted to methane and water in a Sabatier reactor. The methane and water are then reduced to CO and hydrogen which are subsequently combined in the presence of a catalyst to form methanol. The methanol is then reacted with oxygen to form formaldehyde and water. The "formalin" solution is condensed in the presence of a calcium hydroxide catalyst to form a "sugar" solution. The calcium hydroxide catalyst is separated by electrodialysis and recycled. At this point, any toxic components of the carbohydrate mixture are separated, incinerated, and recycled to the system. The edible sugar solution is then processed to remove the excess water. It is assumed that the yield of edible sugars will be 50 percent of the carbohydrate synthesized.

TABLE IX. - VOLUME-WEIGHT BREAKDOWN OF THE HYDROGENOMONAS-ACTIVATE SLUDGE, ANAEROBIC DIGESTION CONFIGURATION

Item no.	Description	Volume, liters	One man, 600 days; weight, -kg					Process heat, W				
			Fixed	Expendables	Power penalty	Heat rejection	Process heat		Total penalty			
1	O ₂ accumulator	40	6									
2	H ₂ accumulator	140	21									
3	H ₂ O storage	40	50									
4	Food storage	20		3								
5	Food processing	100	20	7	0.5	0.4	10	25	15			
6	CO ₂ concentrator	100	16		4.5	12.0	90	800	70			
7	CO ₂ accumulator	20	3									
8	H ₂ O electrolysis	220	55		41	12.3	820	820				
9	H ₂ O recovery	355	45		3.4	11.5	67	767	700			
10	Nutrient feed tank	10	15									
11	Nutrient feed pump				0.1		2	2				
12	Hydrogenomonas reactor	120	120		15.0	4.5	300	300				
13	Suspended solids separator	1	1		0.5	0.4	10	10				
14	Activated sludge effluent pump				0.1		2	2				
15	Activated sludge liquid-solids separator	1	1		0.3	0.1	5	5				
16	Activated sludge reactor	10	10		1.3	0.4	25	25				
17	Activated sludge feed pump				0.1		2	2				
18	Waste collector-pulverizer	280	40		0.7	0.2	13	13				
19	Activated sludge gas-liquid separator	1	1		0.3	0.1	5	5				
20	Anaerobic reactor	40	10		6.0	1.8	120	120				
21	Gas-liquid separator	1	1		0.3	0.1	5	5				
22	Combustor	1	1			2.9	190	190				
23	Effluent gas conditioner	1	1									
24	Liquid-solids separator	1	1									
25	Sludge solids storage	15										
26	Anaerobic effluent pump				0.1		2	2				
27	Anaerobic gas pump				0.1		2	2				
	Subtotal	1517	418	10	74.3	46.7	1480	3095	1425	548.8		
	20 percent plumbing instrumentation	303	84		14.9	9.3	296	581	285	107.8		
	Total	1820	502	10	89.2	56	1776	3676	1710	683		
	Adjustments	-134								-151		
	Adjusted total	1686								532		

TABLE X. - AMINO ACIDS IN HUMAN DIET

Description	Amount g/man-day
L-Arginine-HCl	4.38
L-Histidine-HCl-H ₂ O	2.68
L-Isoleucine	4.10
L-Leucine	6.50
L-Lysine-HCl	6.09
L-Methionine	2.97
L-Phenylalanine	2.97
L-Threonine	4.10
L-Tryptophan	1.27
L-Valine	4.52
L-Alanine	4.38
L-Asparagine	---
L-Aspartate	9.33
L-Cysteine ethyl ester-HCl	1.52
L-Glutamate, monosodium	8.83
L-Glutamine	11.50
Glycine	2.84
L-Proline	17.50
L-Serine	9.05
L-Tyrosine ethyl ester-HCl	11.60
Total weight	116.13 g/man-day

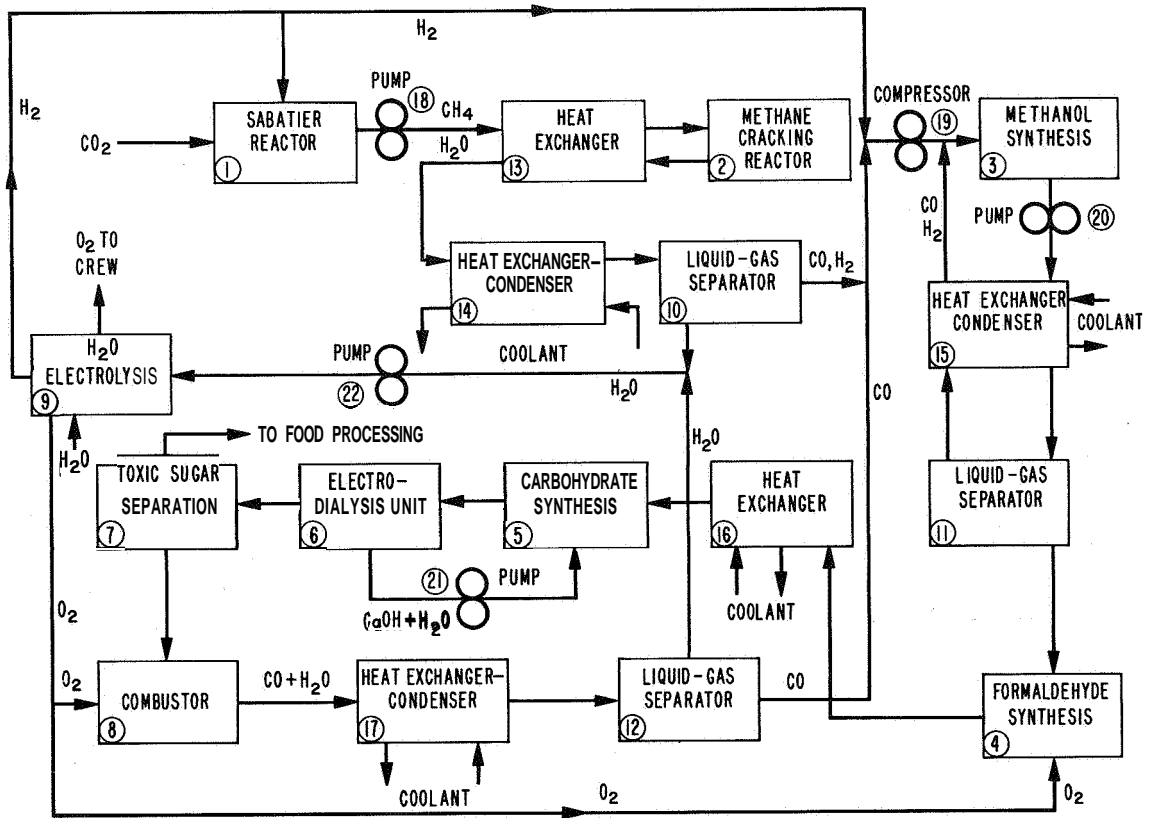


Figure 24.- Carbohydrate synthesis system 1.

TABLE XI. - CARBOHYDRATE SYNTHESIS SYSTEM 1

[Basis: 10 men, 600 days]

Component no.	Component description	Hardware				Performance			Power, kW	Heat rejection, kW
		Volume, liters	Hardware	Power penalty	Heat rejection	Total penalty				
1	Sabatier reactor	19.0	8.9	*	6.3	15.2	*	0.417		
2	Methane cracking reactor	8.5	10.9	30.9	7.6	49.4	0.615			
3	Methanol synthesis reactor	11.3	35.4	*	8.8	44.2	*	0.583		
4	Formaldehyde synthesis	8.5	6.4	*	9.3	15.7	*	0.621		
5	Carbohydrate synthesis	9.1	6.8	*	---	6.8	*	---		
6	Electrodialysis unit	396.0	159.0	82.7	24.9	266.6	1.660	1.660		
7	Toxic sugar separator	116.0	66.0	32.7	7.0	105.7	0.650	0.3340		
8	Toxic sugar combustor	86.6	27.9	6.8	2.0	36.7	0.135	0.825		
9	Electrolysis unit	424.0	114.5	90.0	10.3	214.8	1.800	0.685		
10	Liquid-gas separator	2.8	0.7	---	---	0.7	---	---		
11	Liquid-gas separator	2.8	18.6	---	---	18.6	---	---		
12	Liquid-gas separator	2.8	0.7	---	---	0.7	---	---		
13	Heat exchanger	0.3	0.1	---	---	0.1	---	---		
14	Heat exchanger-condenser	0.3	0.1	---	---	0.1	---	---		
15	Heat exchanger-condenser	4.0	22.3	---	---	22.3	---	---		
16	Heat exchanger	0.3	0.1	---	---	0.1	---	---		
17	Heat exchanger-condenser	0.3	0.1	---	---	0.1	---	---		
18	Pump, feed, CH ₄	1.7	2.3	0.5	Neg.	2.8	0.010	Neg.		
19	Compressor	27.2	31.8	18.2	1.0	51.0	0.360	0.063		
20	Pump, recycle, CO, H ₂	1.4	5.90	0.3	Neg.	6.2	0.005	Neg.		
21	Pump, recycle, CaOH	1.4	2.2	0.3	Neg.	2.5	0.005	Neg.		
22	Pump, H ₂ O	1.4	2.2	0.3	Neg.	2.5	0.005	Neg.		
	Component subtotal	1125.7	522.9	262.7	77.2	862.8	5.245	5.803		
	Plumbing, instrumentation, and controls	114.3	68.3	28.0	12.0	108.3	0.559	0.817		
	Component total	1240	591.2	290.7	89.2	971.1	5.804	6.620		
	System total	3683.2	591.2	290.7	89.2	971.1	5.804	6.620		

*Power required during start-up of reactor only.

TABLE XII. - CARBOHYDRATE SYNTHESIS SYSTEM 2

[Basis: 10 men, 600 days]

Component no.	Component description	weight penalty, kg				Volume, liters		weight penalty, kg		Heat rejection, kW	Power, kW	Heat rejection, kW
		Hardware	Power penalty	Heat rejection	Total penalty	Power penalty	Heat rejection					
1	CO ₂ electrolysis unit	262	180	20.6	184.0	180	20.6	462.6	3.61	1.373	1.373	
2	CO ₂ concentrator	106.8	25.0	7.5	286.0	25.0	7.5	139.3	0.50	0.500	0.500	
3	Methanol synthesis reactor	35.4	*	8.8	11.3	*	8.8	44.2	*	0.583	0.583	
4	Formaldehyde synthesis	6.4	*	9.3	8.5	*	9.3	15.7	*	0.621	0.621	
5	Carbohydrate synthesis	6.8	*	Neg.	9.1	*	Neg.	6.8	*	Neg.	Neg.	
6	Electrodialysis unit	159.0	82.7	24.9	396.0	82.7	24.9	266.6	1.66	1.660	1.660	
7	Toxic sugar separator	66.0	32.7	7.0	116.0	32.7	7.0	105.7	0.650	0.3340	0.3340	
8	Toxic sugar combustor	27.9	6.8	2.0	86.5	6.8	2.0	36.7	0.14	0.135	0.135	
9	H ₂ O electrolysis unit	114.5	90.0	10.3	425.0	90.0	10.3	214.8	1.80	0.685	0.685	
10	Liquid-gas separator	0.7	---	---	2.8	---	---	0.7	---	---	---	
11	Liquid-gas separator	18.6	---	---	2.8	---	---	18.6	---	---	---	
12	Heat exchanger	0.1	---	---	0.3	---	---	0.1	---	---	---	
13	Heat exchanger-condenser	22.3	---	---	4.0	---	---	22.3	---	---	---	
14	Heat exchanger	0.1	---	---	0.3	---	---	0.1	---	---	---	
15	Heat exchanger-condenser	0.1	---	---	0.3	---	---	0.1	---	---	---	
16	Pump, recycle, CO ₂	2.2	0.5	Neg.	1.4	0.5	Neg.	2.7	0.01	Neg.	Neg.	
17	Compressor	31.8	18.2	1.0	27.2	18.2	1.0	51.0	0.36	0.063	0.063	
18	Pump, recycle, CO ₂ , H ₂	5.9	0.3	Neg.	1.4	0.3	Neg.	6.2	0.005	Neg.	Neg.	
19	Pump, recycle, CaOH	2.2	0.3	Neg.	1.4	0.3	Neg.	2.5	0.005	Neg.	Neg.	
20	Pump, H ₂ O	2.2	0.3	Neg.	1.4	0.3	Neg.	2.5	0.005	Neg.	Neg.	
21	Component subtotal Plumbing, instrumentation, and control	871.0	436.8	91.4	1565.7	436.8	91.4	1399.2	8.745	5.954	5.954	
	Component total	138.0	63.2	14.8	205.0	63.2	14.8	216.0	1.260	0.985	0.985	
	System total	1009.0	500.0	106.2	1770.7	500.0	106.2	1615.2	10.005	6.939	6.939	
		1009.0	500.0	106.2	6250	500.0	106.2	1615.2	10.005	6.939	6.939	

*Power required during start-up of reactor only.

Engineering Evaluation of Composite Subsystems, Biological and Physicochemical

Combining physicochemical and biological subsystems of the closed food-waste cycle offers an opportunity to circumvent some of the deficiencies of each type. Selection of subsystems is in accord with the following reasoning:

1. Since physicochemical synthesis of fats and amino acids is non-competitive, only carbohydrates are synthesized in the composite system.
2. The culture of *Hydrogenomonas* is under near optimum conditions of nitrogen/ CO_2 ratio and will utilize all of the nitrogen recovered from man's wastes. Under such conditions it is capable of using about 20 percent of the CO_2 . Balance is achieved by using whatever CO_2 remains in carbohydrate synthesis.
3. The choice of waste processing method provides maximum recovery of nitrogen in forms assimilated by *Hydrogenomonas*. This is an activated sludge subsystem, with recycle of suspended solids through an ultrasonic unit for physical degradation of particles and bacterial cells.

Figure 26 is a diagram of the composite configuration. Weight and volume summaries are in table XIII. An important characteristic of the composite

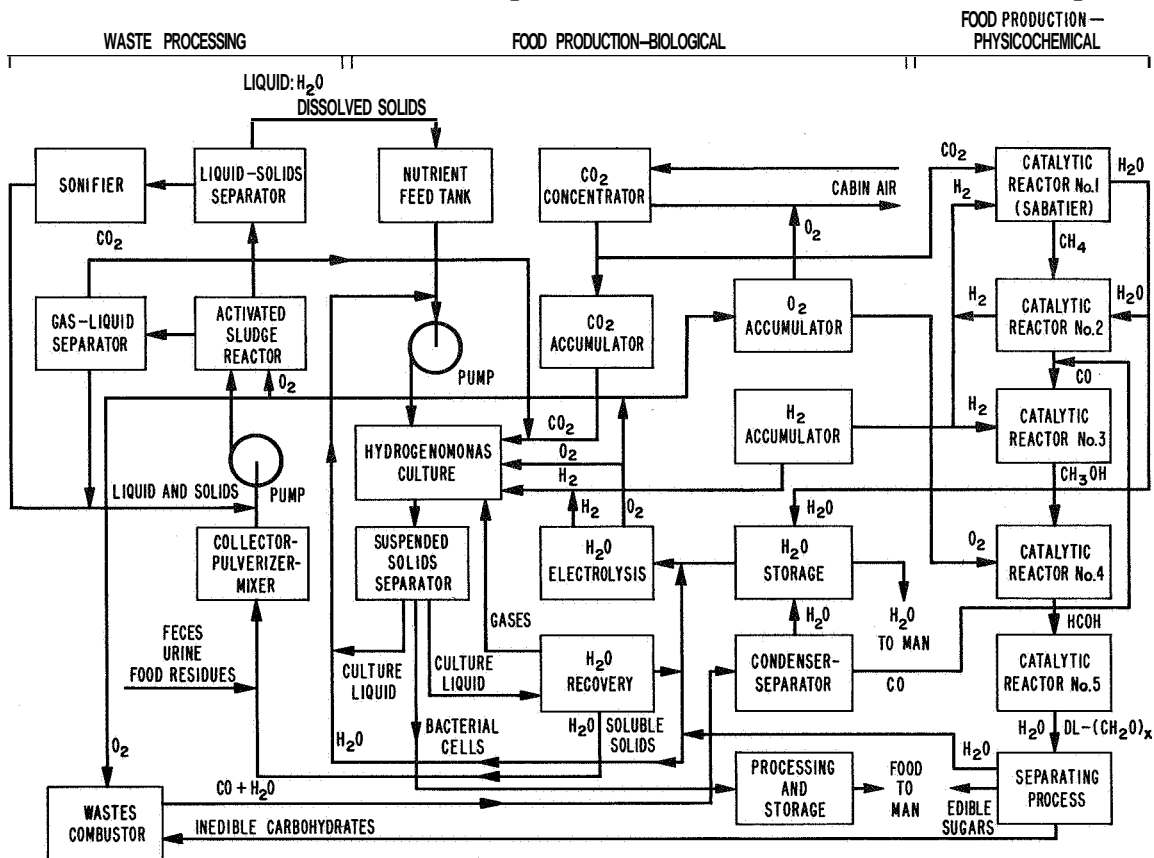


Figure 26.- Composite subsystems food-waste cycle.

TABLE XIII. - WEIGHT AND VOLUME ESTIMATES; COMPOSITE FOOD-WASTE CYCLE

Description	Volume, liters	Power, W	Process heat, W	Heat rejection, W	Weight, kg				Total
					Fixed	Expendable	Power	Process heat	
O ₂ accumulator	40				6				
H ₂ accumulator	140				21				
H ₂ O storage	40				50				
Food processing	20	2	3	5	4	2			
CO ₂ concentrator	100	90	710	800	16				
CO ₂ accumulator	20				6				
H ₂ O electrolysis	177	661		275	44				
H ₂ O recovery	71	14	140	154	9				
Nutrient feed tank	2				3				
Hydrogenomonas reactor	24	60		60	24				
Suspended solids separator	1	5		5	1				
Activated sludge effluent pump		2		2					
Liquid-solids separator	1	3		3					
Activated sludge feed pump		2		2					
Waste collector-pulverizer	280	13		13	40				
Sonifier	1	7		7	1				
Gas-liquid separator	1	3		3	1				
Sonifier effluent pump		2		2					
Nutrient feed pump		2		2					
Activated sludge reactor	10	25		25	10				
Plumbing, instrumentation, controls	186	178		178	47				
Carbohydrate synthesis	297	400		494	48				
Total	1411	1469	853	2030	331	2	74	13	31
Adjustment	-134								
Adjusted total	1277								451
									-151
									300

configurations is that it makes available some alternate modes of operation. Failure of either the Hydrogenomonas culture or the carbohydrate synthesis subsystem is only a partial failure in food supply. In the normal mode of operation, carbohydrate synthesis uses about 80 percent of the CO₂ produced. If this subsystem were made slightly oversize, it could handle all of the CO₂ and would be operated in this manner during malfunction of the Hydrogenomonas culture. This alternate mode would prevent loss of CO₂ and would provide the total calories of the diet. Only the protein component of the diet would be lost during the malfunction.

To some extent, a similar alternate mode is available during loss of the carbohydrate synthesis subsystem. Excess CO₂ would be fed into the Hydrogenomonas culture, and its uptake would depend on culture conditions such as culture volume, bacterial cell density, and the inorganic nutrient supply. Although the nitrogen/CO₂ ratio would be less favorable than initially, the culture conditions could be designed to increase the cell production and harvest rate to partially compensate for loss of the synthetic carbohydrates.

Normal mode operation of the composite configuration has additional advantages in comparison with either single technique. A more varied diet is possible, including some variation in the protein/carbohydrate ratio. It is expected that this characteristic will bring a higher degree of crew acceptance. Another advantage is that the flexibility in use of CO₂ makes it possible to approach optimum culture conditions for the Hydrogenomonas. This minimizes weight, volume, and power requirements.

Qualitative Evaluation of Noncompetitive Configurations

The configurations discussed in this section are categorized as noncompetitive. Their characteristics were presented in preceding sections and are summarized in the following paragraphs. However, a noncompetitive technique, as judged by the present level of knowledge, may prove to be a preferred technique as knowledge expands. There may also be unusual systems constraints which would bias a selection. Therefore, a designation as noncompetitive is not intended as a recommendation that the technique or configuration be permanently discarded.

Algae.- An algal system has general similarities to a hydrogen bacteria system in that: (1) the biomass produced as a food material is too rich in protein and is deficient in carbohydrate; (2) there is a nitrogen balance problem due to insufficient nitrogen in human wastes for producing biomass in the quantity necessary for nutrition of man; (3) inorganic salts are excreted by man in greater quantity than utilized by the microorganisms; and (4) there are engineering problems in mixing and in separations of the solid, liquid, and gaseous components of the culture. The differences of consequence, which make an algal system noncompetitive, are:

1. Algal suspensions must be in thin layers for efficient light utilization. This geometric constraint means that the algal system must have considerably greater weight and volume than a hydrogen bacteria

system since the liquid volume of the algal culture will be at least will be at least as great as that of the hydrogen bacteria culture.

2. The algal system is burdened with inefficient utilization of light energy by the algae and by inefficient conversion of electrical energy to light energy. The electrical power requirement is estimated at 10 kW/man, in comparison with a hydrogen bacteria system at about 1 kW/man.
3. The heat rejection requirement is almost in direct proportion to the electrical input, so that an algae system compares unfavorably with hydrogen bacteria by a factor of about 10.

A hydrogen bacteria system requires auxiliaries for CO₂ concentration from cabin air and for water electrolysis. These requirements have been included in the foregoing comparison of algae with Hydrogenomonas. Therefore, it is concluded that the algal system offers no significant advantages and has substantial disadvantages in comparison with Hydrogenomonas.

Higher plants. - There may be justification for a higher plants culture which supplies a very small fraction of the food for man. However, a culture which supplies such food is considered noncompetitive for reasons generally like those regarding the algal cultures:

1. Geometric constraints inherent in light contact lead to relatively large weight and volume.
2. The electrical power requirement for lighting is exorbitant. An input of 10.8 kW/man was estimated for providing only 30 percent of the total dry food requirement.
3. The heat rejection requirement is very high, in accord with the high electrical input.

Higher plants have some additional disadvantages in comparison with other systems: (1) a significant fraction of the plant is inedible, which burdens the waste-processing system, (2) the growth cycle is long so that periodic food harvest would involve preservation and storage to allow for unmatched consumption and production, and (3) leaf transpiration would load the cabin atmosphere with about 400 lb/man-day of water vapor. This would impose an enormous burden on the cabin air-conditioning system.

Fungi (mushrooms). - A mushroom culture is estimated to weigh 3,510x lb, where x is the weight (in lb) of mushrooms produced per day. Since mushrooms have a caloric value of only 40 cal/lb, a mushroom culture is obviously noncompetitive for producing any significant portion of man's caloric intake.

Physicochemical synthesis of fats and amino acids. - There is no significant technology for physicochemical synthesis of fats from the raw materials of human wastes, which in itself is justification for a noncompetitive rating. The complex molecular structures of fats (relative to other materials

considered for food synthesis, such as CO_2 , CO , H_2O , CH_4 , CH_3OH , and HCOH) suggest correspondingly that a complex series of process steps would be involved in physicochemical synthesis.

While all the amino acids have been synthesized, the technology is non-competitive in comparison with other methods for supplying protein or protein derivatives for man's diet. Amino acid yields by pansynthesis have been extremely low. Industrial synthesis of individual amino acids has not been constrained in choice of raw materials to those readily available in man's wastes.

Animal cultures. - Life support requirements for mammals are similar to those for man. An animal colony would place added burdens on systems for processing wastes and for producing food and water. Animal flesh may be more suitable nutritionally and psychologically as human food than is the biomass derived from a bacterial culture. An upgrading of food quality would be achieved by feeding the biomass to animals and "harvesting" the animals for man's food. If animals show a total efficiency of metabolizable energy of 30 percent (ref. 92), and if two-thirds of the animal body mass is consumed as food, the upgrading has an efficiency of only 20 percent. Thus, the penalties to the spacecraft systems would be five times higher than if the biomass is consumed directly by the crew. In addition, animal cultures in zero gravity would present severe problems in feeding and sanitation. Although some of these problems might be circumvented by raising herbivorous fish in an algae suspension, the basic inefficiency would remain.

CONCLUSIONS

This study has been a necessary step in achieving a master plan for developing a closed ecology. Evaluations were made among potentially competitive configurations and were necessarily based on inadequate technology. Rapid advances are to be expected in the biological and physical sciences. As a consequence, these results must be considered as preliminary. In the absence of abundant information resources, they offer at least a reasonable basis for channeling subsequent work toward the goal of supporting manned space missions of long duration.

Major Areas of Concern

The subsystem and food-waste loop studies documented in the previous sections have presented numerous areas for potential research and development. Regardless of the system ultimately recommended for the synthesis of food in the closed ecology, areas that will certainly require some portion of the research and development effort can be defined. There are two significant areas with a high probability of future research and development effort. These pertain to the nutritional aspects of the food-waste loop and the ultimate acceptance or rejection of the produced food by the crew.

Nutrient balance.- Using cultures of microorganisms for closing of the food-waste loop poses problems in balancing nutritional component synthesis with dietary requirements of the crew. For example, the Hydrogenomonas culture can use the total nitrogen contained in wastes of the crew but in so doing will only consume about 20 percent of the CO₂ produced by the crew. This leads to a requirement for additional means to convert the remaining CO₂ to nutrients for the crew.

Nutrients from waste.- The major portion of man's metabolic wastes is distributed in the expired air from the lungs, the urine, and the feces. Probably the least accessible and least useful waste products are those remaining in the feces after the water has been removed. The recovery of usable nutrients from dry feces requires potentially complex techniques. The total dry feces weight is only 3.5 percent of man's metabolic waste output. The recovery of this small quantity of waste and processing to usable nutrients may not be reasonable compensation for the potential system penalties and complexity.

Nutritional value of biomasses. - The use of biomasses for a substantial part of a space crew's food allotment is possibly one of the most important areas which needs investigation. Of the potentially useful biological methods for food production, only the algae systems have had any significant human feeding experiments. It appears from these tests that there may be limitations on the amount of algae that man can reasonably consume to provide proper nutrition. The use of Hydrogenomonas, which appears from the engineering standpoint to offer a more efficient means of producing food than algae, has had almost no human feeding experiments and little is known about its true nutritional value to man.

Crew acceptance.- The crew acceptance of the synthesized food may well prove to be the major restraint in this method of closing the ecology. The optimum solution of the biological, chemical, physical, and engineering problems and final synthesis of food from waste will not guarantee acceptance by the crew.

REFERENCES

1. Staff of Life Support Project, Life Sciences Dept.: Life Support System for Space Flights of Extended Time Periods, Final Report: Program Summary and Test Results. Report No. 64-26234, General Dynamics, Convair Division. Prepared for NASA/Langley Research Center, Contract NAS 1-2934, December 1965.
2. Ehricke, K. A., et al.: A Study of Early Manned Interplanetary Missions. Final Summary Report. Report AOK 63-0001, General Dynamics/Astronautics. Prepared for George C. Marshall Space Flight Center, Contract NAS 8-5026, 31 January 1963.
3. Myers, J., et al.: Study of a Photosynthetic Gas Exchanger. Tech. Doc. Report SAM-TDR-64-52, 1964.
4. Zuraw, E. A., et al.: Photosynthetic Gas Exchanger Performance at High Light Input, Contract NASw-95, Report No. U413-63-036, General Dynamics Electric Boat, 1963.
5. Hannan, P. J., et al.: A Study of the Feasibility of Oxygen Production by Algae for Nuclear Submarines. NRL Report 5954, U. S. Naval Research Laboratory, 1963.
6. Gaucher, T. A., et al.: Mass Propagation of Algae for Photosynthetic Gas Exchange. Bioch. Microbiol. Tech. Engr., vol. 2, 1960, pp. 339-359.
7. Hemerick, G., et al.: Engineering Research on a Photosynthetic Gas Exchanger. ONR Contract NOrn 3394(00), Report U413-62-018, General Dynamics Electric Boat, 1962.
8. Bovee, H. H., et al.: Large Algal Systems, Tech. Doc. Report AMRL-TDR-62-116, 1962, pp. 8-19.
9. Myers, J.: Study of a Photosynthetic Gas Exchanger as a Method of Providing for the Respiratory Requirements of the Human in a Sealed Cabin. SAM Report 58-117, 1958.
10. Thacker, D. R., et al.: The Mass Culture of Algae. J. Solar Energy Science and Engineering, vol. 1, 1957, pp. 37-50.
11. Newland, R. G., et al.: Design Study of Gravity-Independent Photosynthetic Gas Exchanger. Tech. Doc. Report AMRL-TDR-63-59, 1963.
12. Gafford, R. D., et al.: Solar Illuminated Photosynthetic Gas Exchangers. Tech. Doc. Report AMRL-TDR-62-116, 1962, pp. 42-62.
13. Burlew, J. S., ed.: Algal Culture from Laboratory to Pilot Plant. Publication 600, Carnegie Institute of Washington, 1953.

14. Myers, J. : Culture Conditions and the Development of the Photosynthetic Mechanism. IV - Influence of Light Intensity on Photosynthetic Characteristics of Chlorella. J. Gen. Physiol., vol. 29, 1946, pp. 429-440.
15. Ammann, E., et al. : Gas Exchange of Algae. I - Effects of Time, Light Intensity and Spectral Energy Distribution on the Photosynthetic Quotient of Chlorella pyrenoidosa. Appl. Microbiol., vol. 13, 1965, pp. 546-551.
16. Leone, D. : Photosynthetic Gas Exchange in the Closed Ecosystem for Space. Part II - Studies on the Growth of Thermophilic Chlorella 71105. Contract NASw-95, Report No. U411-61-106, General Dynamics Electric Boat, 1961.
17. Zuraw, E. A., et al. : Photosynthetic Gas Exchange in the Closed Ecosystem for Space. Contract NASw-95, Report No. SPD-90, General Dynamics Electric Boat, 1960.
18. Golueke, C. G., et al. : A Study of Fundamental Factors Pertinent to Microbiological Waste Conversion in Control of Isolated Environments. Contract AF19(628)2462, SERL Report No. 64-5, March 1964.
19. Golueke, C. G. : The Biological Control of Enclosed Environments. Sewage and End Wastes, vol. 31, 1959, pp. 1125-1142.
20. Leone, D. : Biological Waste Treatment in the Closed Ecosystem for Space System. Contract NASw-95, Report No. U41-63-041, General Dynamics Electric Boat, 1963.
21. Pipes, W. O., et al. : Growth of Chlorella on Products from the Incineration of Human Wastes. Tech. Doc. Report AMRL-TDR-62-116, 1962, pp. 315-327.
22. Lubitz, J. A. : Animal Nutrition Studies with Chlorella 71105. Tech. Doc. Report AMRL-TDR-62-116 (Biologistics for Space Systems Symposium), 1962, pp. 331-356.
23. Powell, C. C., et al. : Algae Feeding in Humans. J. Nutrition, vol 75, 1961, pp. 7-12.
24. Leveille, G. A., et al. : Nutrient Content of Various Algae and Amino Acid Adequacy for Growth of Rats and Chicks. Tech. Doc. Report AMRL-TDR-62-116 (Biologistics for Space System Symposium), 1962, pp. 405-419.
25. Vinograd, S. P. : Nutritional Trends in Future Manned Space Flights. Paper presented at Conference on Nutrition in Space and Related Waste Problems (Tampa, Fla.), Apr. 27-30, 1964, pp. 17-21.
26. Nakamura, H. : Chlorella Feed for Animal Husbandry. International Chlorella Union, c/o Dr. Hiroshi Nakamura: 2 Kokoi-cho, Nakano-Ku, Tokyo, Japan, 38 pp., 1964.

27. Vanderveen, J. E., et al.: Nutritional Value of Algae Grown Under Sterile Conditions. Tech. Doc. Report AMRL-TDR-62-116 (Biologistics for Space Systems Symposium), 1962, pp. 357-364.
28. Hegsted, D. M.: Proteins in Space Nutrition. Paper presented at Conference on Nutrition in Space and Related Waste Problems (Tampa, Fla.), Apr. 27-30, 1964, pp. 135-141.
29. Boyko, N. M., et al.: The Use of Unicellular Algae for Human Nutrition. Voprosy Pitaniya (Problems of Nutrition), vol. 22, 1963, pp. 3-8.
30. Wallman, H., et al.: Design and Development of a Photosynthetic Gas Exchanger. Tech. Doc. Report AMRL-TDR-62-37, 1962.
31. Anon.: ONR Conference on Photosynthetic Gas Exchangers. Office of Naval Research, 1956.
32. Garrick, L.: The Status of Development of Photosynthetic Gas Exchangers. NASA/Langley Research Center, 1963.
33. Miller, R. L., et al.: Algal Bioregenerative Systems. Environmental Systems Branch, USAF School of Aerospace Medicine (Brooks AFB, Tex.), 1965.
34. Golueke, C. G., et al.: Role of Plants in Closed Systems. Ann. Rev. Plant Physiol., vol. 15, pp. 387-408, 1965.
35. Schlegel, H. G.: Die Verwendung von H_2 - oxydierenden Bakterien zur Regenerierung der Atomluft. Raumfahrtforschung, vol. 8, 1964, pp. 65-67.
36. Canfield, J. H.; and Lechtman, M. D.: Study of Hydrogen-Fixing Microorganisms for Closed Regenerating Biosystems. Tech. Doc. Report AMRL-TDR-64-34, June 1964.
37. Bongers, L.; and Kok, B.: Life Support Systems for Space Missions. Develop. in Ind. Microbiol., vol. 5, 1964, pp. 183-195.
38. Foster, J.; and Litchfield, J.: Microbial Utilization of Hydrogen Produced by Electrolysis of Water in Closed-Cycle Space Systems. Paper presented at 53rd National Meeting, Amer. Inst. of Chem. Engineers (Pittsburg, Pa.), 18 May 1964. (Biotech. and Bioeng., vol. 6, 1964, pp. 441-456)
39. Bongers, L.: Sustaining Life in Space - A New Approach. Aerospace Medicine, February 1964, pp. 139-144.
40. Bongers, L.: Chemosynthetic Gas Exchanger. Contract NASw-713, Report No. ERL3270-4, 1964.
41. AFHMD-TR-16 (XII): SR 192, Strategic Lunar system, Vol. XII.

42. Anon.: Investigation of Selected Higher Plants as Gas Exchange Mechanisms for Closed Ecological Systems. AMRL-TDR-62-127, Boeing Co., September 1962.
43. Moss, D.: Plant Leaves for the Production of Oxygen in a Closed System. NAS4-129, Conn. Agr. Expt. Station (New Haven, Conn.).
44. Beu, D. E.: The Use of Higher Plants as Components in a Life Support System. Battelle Memorial Institute (Columbus, O.), 1965.
45. Leone, D. E.: Biological Waste Treatment in the Closed Ecosystem for Space Systems. Contract NASw-93, Report No. U413-63-041, General Dynamics Electric Boat, Jan. 1963.
46. Benishek, K. B., et al.: Effects of Simulated Aerospace System Environments on the Growth of Selected Angiosperms. AMRL-TDR-63-131, Boeing Co., December 1963.
47. Wilks, S.: Preliminary Report on the Photosynthetic Gas Exchange Potentialities of the Family Lemnaceae (Duckweed). Tech. Doc. Report AMRL-TDR-63-131, 1962, pp. 265-278.
48. Wilks, S.: Plant Systems as Long-Term Flight Nourishment Sources. Paper presented at Conference on Nutrition in Space and Related Waste Problems (Tampa, Fla.), 1964.
49. Tolecke, W.: Research on Tissue Cultures of Higher Plants. Tech. Doc. Report AMRL-TDR-63-124, 1963.
50. Tolecke, W.: Growth of Tissues of Higher Plants in Continuous Liquid Culture and Their Use in a Nutritional Experiment. Tech. Doc. Report AMRL-TR-65-101, 1965.
51. Byrne, A., et al.: Food Production by Submerged Culture of Plant Tissue Cells. Science, vol. 135, 1962, pp. 215-216.
52. Bieberdorf, F. W.: A Study of Microbiological Waste Treatment Techniques. Tech. Doc. Report AMRL-TDR-62-142, November 1962.
53. Pipes, W. O.: Waste-Recovery Processes for a Closed Ecological System. National Academy of Sciences Publication 898, 1961.
54. Ingram, W. T.: Microbiological Waste Treatment Processes in a Closed Ecology. Tech. Doc. Report AMRL-TDR-62-126, 1962.
55. Zuraw, E. A., et al.: Closed Water Cycles and Closed Air Revitalization Systems for Deep Diving Submarines. Report No. U413-63-098, General Dynamics Electric Boat, June 1963.

56. Ott, C. : Waste Management for Closed Environments. Paper presented at Conference on Nutrition Related Waste Problems (Tampa, Fla.) , April 1964, pp. 97-102.
57. Bogan, R. H., et al.: Aerobic Biological Degradation of Human Waste in Closed Systems. Paper presented at 6th National Meeting of the American Astronautical Society (New York, N. Y.), 18-20 Jan. 1960.
58. Pote, H. L. : Waste Conversion by Anaerobic Thermophilic Methods. Tech. Doc. Report AMRL-TDR-62-116, 1962, pp. 290-294.
59. Sims, W. L. : OSA Bulletin No. 25. Agriculture Extension Service, University of California.
60. Anon. : Encyclopedia of Science and Technology. Vol. VIII. McGraw Hill Book Co., Inc.
61. Encyclopedia Britannica. Vol. XVI.
62. Tischer, R. G. : Biochemical Study of Mixed Culture Prototype in a Closed Ecological System. NASA Grant NSG 80-60, Progress Report, 1 June 1960 - 31 May 1961.
63. Ibid.: Progress Report IV, 1 January 1962 - 1 July 1962.
64. Ibid.: Progress Report V, 1 July 1962 - 1 December 1962.
65. Gray, W. D.: Fungi as a Nutrient Source. Tech. Doc. Report AMRL-TDR-62-116, 1962, 365-381.
66. Litchfield, J. H., et al.: Nutrient Content of Morel Mushroom Mycelium: Amino Acid Composition of the Protein. J. Food Science, vol. 28, 1963, pp. 741-743.
67. Chandler, H. : Carbon Dioxide Reduction System. AMRL-TDR-64-42, May 1964.
68. Emmett, P. H.: Hydrogenation and Dehydrogenation. Vol. III of Catalysis. Reinhold Pub. Corp. (New York) , 1955.
69. Emmett, P. H.: Oxydation, Hydration, Dehydration, and Cracking Catalysts. Vol. VII of Catalysis. Reinhold Pub. Corp. (New York) , 1960.
70. Akerlof, G. C.: Feasibility of Regeneration of Carbohydrates in a Closed Circuit Respiratory System. J. Spacecraft and Rockets, vol. 1, no. 3, May-June 1964, pp. 303-310.
71. Anon. : Oxygen Recovery System Evaluation for Space Flights of One Year Duration. Contract NAS 1-2934, Report No. 64-26203, General Dynamics, Convair Division. Prepared for NASA/Langley Research Center, 16 Oct. 1964.

72. Conant, J. B. ; and Blatt , A. H. : The Chemistry of Organic Compounds. Macmillan Co. (New York) , 1948.
73. Akerlof, G. C.; and Mitchell, P. W. D.: A **Study** of the Feasibility of the Regeneration of Carbohydrates in a Closed Respiratory System. Final Report, NASA Contract NASr-88, 1 March 1963.
74. Fox, Sidney W. : The **Outlook** for Synthetic Foods. Food Technology, April 1963.
75. Miller, S.: Production of Some Organic Compounds Under Possibly Primitive Earth Conditions. J. Am. Chem. Soc., vol. 77, 1955, P. 2351.
76. Groth, W.: Angew Chem., vol. 69, 1957, P. 681.
77. Terenin, A. N.: Reports of the Moscow Symposium on the Origin of Life. August 1957, P. 97.
78. Groth, W. E.; and Ewysenhoff, H. V.: Photochemical Formation of Organic Compounds from Mixtures of Simple Gases. Planetary Space Science, vol. 2, pp. 79-85.
79. Dose, K.; and Rajewsky, B.: Biochim. et Biophys. Acta, vol. 25, 1957, p. 225.
80. Zimmerman, F. J. : New Waste Disposal Process. Chem. Engineering, 25 Aug. 1958, pp. 117-120.
81. Anon. : Process Consumes Sludge by Flameless Combustion. Chem. Engineer^{ing}, 28 Nov. 1960, pp. 80-82.
82. Tomsic, C. M.; and Zeff, J. D. : Waste Disposal for Aerospace Missions, Tech. Doc. Report AMRL-TDR-63-4, January 1963.
83. Macklin, M.: Waste Combustion and Water Recovery System. J. Spacecraft and Rockets, vol. 1, no. 3, May-June 1964.
84. Anon. : Water Management System Evaluation for Space Flights of One Year Duration. Report No. 64-26206, General Dynamics, Convair Division. Prepared for NASA/Langley Research Center, Contract NAS 1-2934, 29 January 1965.
85. Anon. : Waste Management System Evaluation for Space Flights of One Year Duration. Report No. 64-26205, General Dynamics, Convair Division. Prepared for NASA/Langley Research Center, Contract NAS 1-2934, 12 September 1963.
86. Anon. : Oxygen Recovery System Evaluation for Space Flights of One Year Duration. Report No. 64-26203, General Dynamics, Convair Division. Prepared for NASA/Langley Research Center, Contract NAS 1-2934, 16 October 1964.

87. Anon. : Atmospheric Contaminant Control Subsystem Evaluation for Space Flights of One Year Duration. Report No. 64-26204, General Dynamics, Convair Division. Prepared for NASA/Langley Research Center, Contract NAS 1-2934, 25 August 1964.
88. Bongers, L. : Chemosynthetic Gas Exchanger. Final Report, Period June 1963 through June 1964. Report No. ER 13270-4, prepared by the Martin Co. under NASA Contract NASw-713, July 1964.
89. Anon. : Study of Hydrogen-Fixing Microorganisms for Closed Regenerating Biosystems. Tech. Doc. Report No. AMRL-TDR-64-35, Biomedical Lab., Aerospace Medical Research Labs., Aerospace Medical Division, AFSC (WPAFB, O.), June 1964.
90. Rosenthal, Norman A., et al. : Preliminary Report on Evaluation of a Chemically Defined Liquid Diet Based on the Statistical Analysis of Clinical Data Obtained from Urine Specimens Generated During Human Feeding Trials at the California Medical Facility, Vacaville, California, January 1, 1964 - March 3, 1964. Contract NASw-1087, Schwarz Bioresearch, Inc. , September 1965.
91. Hodgman, C. D.; Weast, R. C.; and Selby, S. M. : Handbook of Chemistry and Physics. Third ed., Chemical Rubber Publishing Co., 1958.
92. Kleiber, M.: Animal Food for Astronauts. NASA SP-70. Paper presented at Conference on Nutrition in Space and Related Waste Problems, 27-30 April 1964.

STUDY OF LIFE SUPPORT SYSTEMS FOR SPACE MISSIONS

EXCEEDING ONE YEAR IN DURATION*

Edited by R. B. Jagow and R. S. Thomas

Lockheed Missiles and Space Company
Sunnyvale, Calif.

The relatively long missions of future manned space flights require the development of regenerative life support systems that minimize as much as possible the expendable materials carried in the spacecraft. The ultimate objective of such developments is a completely regenerative system. The study covered by this report is part of an overall program to develop a closed spacecraft ecological system capable of supporting human life for periods exceeding one year without the need of resupply. The objectives of the study were to survey developments of both biological and physicochemical life support systems; to configure several closed ecological systems; to select preferred systems; to establish critical research and development areas for the selected systems; to plan the research and development effort; and to plan subsequent phases dealing with engineering, fabrication, and evaluation of such a system.

During the first four months of the program, major emphasis was placed on the establishment and comparison of candidate approaches to life support in three categories; physicochemical systems with stored food, chemical synthesis of food, and biological systems.

Considerable data were available on the physicochemical systems and, therefore, the state-of-the-art survey of these systems was completed early in the study. Weight, volume, and power estimates, schematics, and a description of each subsystem were prepared. Comparisons were then made and the candidate approaches to each subsystem function - CO₂ removal, CO₂ reduction, O₂ generation, water reclamation, waste processing, and contaminant control - were rated. The selected subsystems were then combined into two complete physicochemical systems with stored food.

The study of potential methods for chemical synthesis of food was more basic. The nutritional requirements of synthetic food were established and the various synthesis methods were studied to determine the potential yields, reaction rates, and reaction conditions. Glycerol, ethanol, and fructose emerged as promising chemical synthetic foods. Process system characteristics were established for these systems; and weight, volume, and power estimates were made. The chemical synthesis subsystems were then combined with the appropriate physicochemical subsystems to complete the life support system.

Because of the scarcity of data on all but a few biological approaches to life support, the comparison of biological systems was much more difficult than for physical-chemical systems. Algal, Hydrogenomonas, and duckweed

*This work was prepared under contract no. NAS 2-3012.

systems were studied in detail; and engineering estimates were made to allow comparison with the physicochemical systems using stored or chemically synthesized food. A cursory review of other less studied biological approaches, such as those using organisms that utilize methane, acetylene, formaldehyde, glycerol, or carbon monoxide, was conducted; and the results are presented.

The last two months of the study were directed toward comparison of the physicochemical systems using stored food, the physicochemical systems using chemically synthesized food, and the biological systems; the rating of these systems; the identification of research and development problem areas; the preparation of research and development specifications for work in these problem areas, and the planning of phases subsequent to the research and development work.

The more significant ground rules established at a prestudy conference between Lockheed Missile and Space Company and NASA are presented below:

Mission duration	1 to 3 years
Crew size	10 to 100 men
Gravity field	Zero and partial gravity
Vehicle orientation	No specific orientation allowed
Power source	Nuclear
Cabin environment	
Gas composition	Air
Pressure	14.7 psia
Leakage	Zero

The performance of this contract was greatly facilitated by the cooperation of the following people and agencies who provided information on the latest developments in biological and physicochemical systems: J. H. Litchfield and J. F. Foster of Battelle Memorial Institute, R. D. Gafford and M. A. Robinson of Beckman Instruments, J. Meyers of the University of Texas, C. H. Ward and S. S. Wilks of the USAF School of Aerospace Medicine, L. H. Bongers of RIAS, P. J. Hannin of Naval Research Laboratories, H. P. Silverman of TRW Inc., the Research Department of the Wilmot Castle Company, H. M. Tsuchiya of the University of Minnesota, M. Reumont of NASA-MSC, D. Popma, R. Bruce, and W. Hypes of NASA-Langley, and C. G. Roach and E. B. Thompson of Wright-Patterson Air Force Base.

CONCEPT REVIEW AND ANALYSIS

The work presented in this section deals with the survey of candidate system concepts, the analyses of these concepts, and the formulation of several different approaches to meet life support requirements.

PROBLEM STATEMENT

It has always been recognized that the longer the duration of a manned space mission the greater the incentive to recover usable metabolic materials from waste products. While the size of almost all of the remaining spacecraft systems varies only slightly with mission duration, the size of the life support system, if no waste products are reused, is almost directly proportional to mission duration. This means that, while metabolic expendibles constitute only 1 percent of the total payload weight for a two-day mission, they would, with no waste recovery, constitute as much as 60 percent of the total payload for a three-year mission.

In absolute terms, metabolic wastes - mainly evaporative water loss, urine, utility water, expired carbon dioxide, and feces - amount to 10 to 14 lb per man per day. For a 10-man, three-year mission, this amounts to over 100,000 lb without considering the weight of containers.

During the last seven years, substantial laboratory efforts have been devoted to the study of methods of recovering water from the atmosphere and from urine with slightly lesser efforts devoted to the recovery of utility water and to the recovery of oxygen from CO₂. The studies have taken two lines, the biological and the physicochemical. The biological systems have not progressed beyond laboratory models, but the physicochemical system work has led to flight-oriented prototype equipment.

While all the biological systems have inherent capability to manufacture food while performing CO₂ reduction, the food manufacturing aspects of these systems are studied less than the gas exchange characteristics. Only a few abortive attempts have been made to study the chemical synthesis of food from waste in space.

Figure 1 illustrates the rise of life support system weight with increasing mission duration assuming the use of the most efficient presently envi-

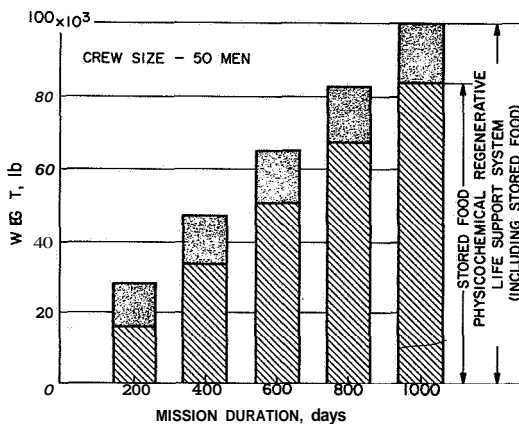


Figure 1.- Relative weight of stored food versus mission duration.

sioned regenerative physicochemical life support system that does not manufacture food. The cross-hatched portion represents the weight of the stored food. Although the figure as drawn refers to a 50 man system, the weight of food, relative to the weight of the remainder of the life support system, is about the same for 5 to 100 man systems for the same mission durations.

Figure 1 shows that, for longer missions, the weight of stored food is as much as 85 percent of the weight of an entire life support system and that, consequently, substantial further weight economies in life support systems can only be attained by lowering the required weight of stored food. The following five methods have been suggested for accomplishing this:

- Manufacture food from wastes biologically
- Manufacture food from wastes chemically
- Use high density food (mass/vol)
- Use high energy density foods (cal/mass)
- Reduce food requirements by reducing metabolism

The manufacture of food from wastes in a spacecraft by biological methods, by physicochemical methods, and by combinations of the two is the main subject of this report and is analyzed in detail following a discussion of physicochemical systems that do not manufacture food.

PHYSICOCHEMICAL SYSTEMS

Candidate Concepts

A large number of candidate approaches have been studied for each of the physicochemical subsystem functions, that is, CO₂ removal, CO₂ reduction, oxygen generation, water reclamation, waste management, contaminant control, and thermal control. Many of these approaches are not competitive for long missions. A list of candidate subsystems that apply to missions greater than one year was prepared early in the study. This list was reviewed with life support personnel at Wright-Patterson Air Force Base, NASA Manned Spacecraft Center, NASA Langley, and NASA Headquarters, as well as several industrial suppliers, to ensure a complete and accurate list. Table I resulted from these discussions.

Subsystem Comparison

The candidate concepts presented in table I were analyzed and a subsystem description; weight, volume, and power estimates; schematics; and a general discussion of the advantages and disadvantages of each subsystem were prepared. This information is presented in the midterm report. For this report, subsystem weight, volume, and power data have been summarized and combined with qualitative estimates of reliability, maintainability, safety, cost, and development effort to allow selection of preferred subsystem approaches. Tables II, III, IV, and V present these data for the four major physicochemical subsystems: CO₂ removal, CO₂ reduction, water electrolysis, and water reclamation. The thermal control, contaminant control, and waste processing subsystems were not considered areas for comparison of candidate approaches. The use of a space radiator system for thermal control and incineration of wastes are probably the only reasonable physicochemical approaches for extremely long space missions. Contaminant control on long missions will probably require the application of almost all the candidate concepts listed in table I.

The weight, volume, and power data tabulated on the subsystem comparison tables were taken from the curves presented in NASA SP-70. The subsystem point ratings represent the qualitative assessment of reliability, maintainability, crew safety, cost, and development. Reliability measures the

TABLE I.- CANDIDATE PHYSICOCHEMICAL SUBSYSTEMS

<p>CO₂ removal</p> <ul style="list-style-type: none">Regenerable molecular sieveElectrodialysisSelective solid absorbent (amine salt)Liquid absorptionCarbonation cell <p>CO₂ reduction</p> <ul style="list-style-type: none">Sabatier reactor plus methane reductionBosch reactorSolid electrolyteFused salt electrolyte <p>Water electrolysis (oxygen generation)</p> <ul style="list-style-type: none">Water vapor cellRotating cellIon exchange membrane cellAbsorbent matrix porous electrode cellCombined electrolysis-vortex separator cell <p>Water reclamation</p> <ul style="list-style-type: none">Distillation - Vapor compressionAir evaporationThermoelectricWaste heat-vacuum <ul style="list-style-type: none">ElectrodialysisElectrolysis <p>Waste disposal</p> <ul style="list-style-type: none">Incineration <p>Contaminant control</p> <ul style="list-style-type: none">SorptionCatalytic burnerFiltersUltraviolet lightIon-discharge conductors <p>Thermal control</p> <ul style="list-style-type: none">Space radiator
--

TABLE II. - COMPARISON OF CANDIDATE CO₂ REMOVAL SUBSYSTEMS

Mission	Subsystem	Weight, lb	Volume, ft ³	Power, W	Weighing factor						Total point count	Rank	
					1-10	1-10	1-10	1-10	1-10	1-10		Wt, Vol, Power	Points
10 men 1 year	Regenerable molecular sieve ^a	500	14	1,500	8	7	9	8	8	40	5	1	
	Electrodialysis	177*	6*	415*	6	6	6	5	5	28	1	4	
	Liquid absorption	317*	5	660	6	6	8	5	4	29	2	3	
	Carbonation cell	153	5	1,690	5	6	7	4	4	26	4	5	
	Selective solid absorbent	377	10	1,125	8	7	8	6	5	34	3	2	
50 men 2 years	Regenerable molecular sieve	2450	57	7,000	8	7	9	8	8	40	5	1	
	Electrodialysis	865*	23*	1,870	6	6	6	5	5	28	1	4	
	Liquid absorption	1220	19	1,850	6	6	8	5	4	29	2	3	
	Carbonation cell	660	28	8,300	5	6	7	4	4	26	4	5	
	Selective solid absorbent	1620	40	5,600	8	7	8	6	5	34	3	2	
100 men 3 years	Regenerable molecular sieve	4150	105	15,000	8	7	9	8	8	40	5	1	
	Electrodialysis	1760 *	39*	3,750	6	6	6	5	5	28	2	4	
	Liquid absorption	2125	34	2,250	6	6	8	5	4	29	1	3	
	Carbonation cell	1750	63	17,000	5	6	7	4	4	26	4	5	
	Selective solid absorbent	3100	79	11,500	8	7	8	6	5	34	3	2	

^a - suitability for oxygen generation capability.

TABLE III.- COMPARISON OF CANDIDATE CO₂ REDUCTION SUBSYSTEMS

Mission	Subsystem	Weight, lb	Volume, ft ³	Power, W	Weighing factor					Total point count	Rank	
					1-10	1-10	1-10	1-10	1-10		Wt, Vol, Power	Points
10 men 1 year	Sabatier reactor plus methane reduction	580*	35*	3,480*	4	4	4	6	4	22	4	3
	Bosch reactor	530*	30*	2,565*	6	5	5	7	6	29	3	1
	Solid electrolyte cell	290	22	2,325	5	4	4	5	4	23	1	2
	Fuzed salt cell	740	46	1,775	3	4	4	4	3	17	2	4
50 men 2 years	Sabatier reactor plus methane reduction	2,580*	155*	18,150*	4	4	4	6	4	2	4	3
	Bosch reactor	1,970*	120*	13,600*	6	5	5	7	6	29	2	1
	Solid electrolyte cell	1,760	135	11,800	5	4	4	5	4	23	1	2
	Fuzed salt cell	4,200	245	8,600	3	4	4	4	3	17	3	3
100 men 3 years	Sabatier reactor plus methane reduction	6,870	* 345*	34,100*	4	4	4	6	4	22	4	3
	Bosch reactor	5,550	* 257*	24,100*	6	5	5	7	6	29	2	1
	Solid electrolyte cell	4,520	272	23,250	5	4	4	5	4	23	1	2
	Fuzed salt cell	10,425	595	17,750	3	4	4	4	3	17	3	4

*Water electrolysis plant is provided to provide common basis of comparison.

TABLE IV.- COMPARISON OF CANDIDATE WATER ELECTROLYSIS SUBSYSTEMS

Mission	Subsystem	Weight, lb	Volume, ft ³	Power, W	Weighing factor					Total point count	Rank	
					1-10	1-10	1-10	1-10	1-10		Wt, Vol, Power	Points
					Rel. Maint.	Crew safety	Cost	Dev.				
10 men 1 year	Water vapor cell	350	2	3,000	7	8	6	6	7	34	4	1
	Rotating cell	605	12	2,650	6	5	3	5	8	27	5	5
	Ion exchange membrane cell	260	3	2,390	6	6	6	7	8	33	3	2
	Absorbent matrix porous electrode cell	140	2	2,350	7	6	6	7	6	32	1	3
	Combined electrolysis vortex separation cell	120	4	2,510	7	6	5	5	7	30	2	4
50 men 2 years	Water vapor cell	1,230	14	15,900	7	8	6	6	7	34	4	1
	Rotating cell	2,150	55	13,500	6	5	3	5	8	27	5	5
	Ion exchange membrane cell	1,270	22	13,100	6	6	6	7	8	33	3	2
	Absorbent matrix porous electrode cell	800	17	12,000	7	6	6	7	6	32	1	3
	Combined electrolysis vortex separation cell	830	27	12,900	7	6	6	5	7	30	2	4
100 men 3 years	Water vapor cell	3,500	42	30,000	7	8	6	6	7	34	4	1
	Rotating cell	4,700	122	26,000	6	5	3	5	8	27	5	5
	Ion exchange membrane cell	2,830	50	24,000	6	6	6	7	8	33	3	2
	Absorbent matrix porous electrode cell	2,360	45	23,250	7	6	6	7	6	32	1	3
	Combined electrolysis vortex separation cell	1,950	59	25,000	7	6	6	5	7	30	2	4

TABLE V.- COMPARISON OF CANDIDATE WATER RECLAMATION SUBSYSTEMS

Mission	Subsystem	Weight, lb	Volume, ft ³	Power, W	Weighing factor						Total point count	Rank	
					1-10	1-10	1-10	1-10	1-10	1-10		Wt, Vol, Power	Points
					Rel. Maint.	Crew safety	Cost	Dev.					
10 men 1 year	Vapor compression distillation	282	12	63	7	5	8	8	8	36	3	1	
	Air evaporation	232	13	525	8	5	8	8	7	36	4	1	
	Thermoelectric distillation	173	9	140	7	6	8	6	6	33	2	3	
	Electrolysis	885	37	52	6	5	7	7	7	32	6	5	
	Electrolysis	131	13	1,925	4	4	4	5	4	21	5	6	
	Waste heat vacuum distillation	173	9	40	7	6	8	7	5	33	1	3	
50 men 2 years	Vapor compression distillation	2,320	50	275	7	5	8	8	8	36	3	1	
	Air evaporation	3,490	135	2,700	8	5	8	8	7	36	4	1	
	Thermoelectric distillation	1,300	55	650	7	6	8	6	6	33	2	3	
	Electrolysis	8,920	340	255	6	5	7	7	7	32	6	5	
	Electrolysis	1,550	90	9,500	4	4	4	5	4	21	5	6	
	Waste heat vacuum distillation	1,240	55	200	7	6	8	7	5	33	1	3	
100 men 3 years	Vapor compression distillation	6,740	112	545	7	5	8	8	8	36	3	1	
	Air evaporation	10,900	365	5,350	8	5	8	8	7	36	5	1	
	Thermoelectric distillation	3,150	145	1,350	7	6	8	6	6	33	2	3	
	Electrolysis	27,400	1,050	475	6	5	7	7	7	32	6	5	
	Electrolysis	4,200	230	19,500	4	4	4	5	4	21	4	6	
	Waste heat vacuum distillation	2,980	155	400	7	6	8	7	5	33	1	3	

relative ability of each system to provide the required function throughout the mission. Maintainability measures the amount and type of maintenance required to keep this system in operation. The safety rating indicates the seriousness of a failure, fire, explosion, contamination, etc. Cost reflects the needs of research, development, engineering, fabrication, and qualification of the system. The development rating measures the magnitude of the development effort other than cost, that is, probability of success, schedule, and availability of special talents, resources, and facilities. The total point count is the sum of the five rated categories. The preferred subsystem concepts were selected on the basis of total point count, weight, volume, and power.

CO₂ Removal.- The electro dialysis, liquid absorption, and selective solid absorbent CO₂ removal systems are all contenders for the selected approach. The regenerable molecular sieve, while having the highest point count, is prohibitively high in weight, volume, and power and the carbonation cell rates poor on all counts. The electro dialysis and liquid absorption systems were selected as the two most promising approaches. The selective solid absorbent system is considerably heavier and requires much more power than the two selected systems and does not have a sufficient point advantage to override these disadvantages.

CO₂ Reduction.- The solid electrolyte cell and Bosch reactor were selected as the CO₂ reduction methods to be included in the integrated physico-chemical system. The solid electrolyte cell is the lightest system and has a reasonably favorable point count. The Bosch has the highest point count with only a small weight penalty relative to the fused salt cell, which rates very poor on point count. The Sabatier reactor plus methane reduction rates relatively poor on both counts, primarily because of the difficulties with methane reduction. The solid electrolyte cell integrates well with the chemical food synthesis systems because it produces CO, which is the preferred food synthesis starting material.

Water electrolysis.- The absorbent matrix porous electrode cell and the ion exchange membrane cell were selected as preferred water electrolysis systems because they represent the best combination of weight, volume, and power and point count rating. With the exception of the rotating cell, the choice was somewhat arbitrary because the four remaining cells were relatively close in total rating.

Water reclamation. - The vapor compression, air evaporation, thermoelectric, and waste heat vacuum distillation systems are very competitive, and selection from these four systems was difficult. The thermoelectric and waste heat vacuum distillation systems were selected because the margins in weight, volume, and power are greater for these systems than the point count margins for the other two approaches. The electrolysis system has a poor rating because of the high power required for the electrolysis process and the reliability problems associated with electrolyzing urine. The electro dialysis system is heavy because of the charcoal and chemicals required to remove urea from the urine before processing.

Subsystem Integration

The selected subsystem approaches were combined into two physicochemical systems to be compared with the other life support systems generated during the study. The combined systems were identified as systems A and B.

Subsystem function	Physicochemical system A	Physicochemical system B
CO ₂ removal	Electrodialysis	Liquid absorption
CO ₂ reduction	Bosch reactor	Solid electrolyte
Water electrolysis	Absorbent matrix	Ion exchange membrane
Water reclamation	Waste heat-vacuum distillation	Thermoelectric distillation
Thermal control	Space radiator	Space radiator
Contaminant control	Burner, sorption, filters	Burner, sorption, filters
Waste disposal	Incineration	Incineration

The electrodialysis and solid electrolyte cell subsystems produce oxygen in the process of removing and reducing CO₂. If they were utilized in the same system, excess oxygen would be produced unless substantial cabin leakage were encountered. Therefore, the electrodialysis and Bosch subsystems were combined in system A and the liquid absorption and solid electrolyte cell subsystems were combined in system B.

The remaining combinations were arbitrary. Weight, volume, and power for these combined systems were estimated during the system comparison studies presented later in the report. The information in tables II through IV was combined with data for the other subsystems, including food supply, to provide total system weight, volume, and power.

THE CHEMICAL SYNTHESIS OF FOOD

The logical extension of the physicochemical regenerative system is the chemical synthesis of food from metabolic and other wastes, such as CO₂ and urine. As mentioned earlier, systems that do not manufacture food must supply about 1-1/2 lb of food per mission man-day. Figure 1, based on the type of system described in the previous section, shows that 85,000 lb of a total 100,000 lb life support system (50 men, 1000 days) was stored food. It is evident that any further substantial lowering of the life support system weight for longer mission must involve the manufacture of food from wastes and that the potential savings from such manufacture of food could decrease the total life support system weight by as much as 85 percent for a three-year mission, and even more for longer missions.

Two possible methods for manufacturing food from wastes in spacecraft have been considered, namely, biological and chemical. Biological synthesis has received much more study than chemical synthesis and recommends itself more instinctively since it is the method utilized by our own earth ecology

and, potentially, is capable of reproducing the entire spectrum of steaks, chops, and vegetables with which we are familiar. In practice, however, the production of such familiar foods in space would require a system of enormous complexity with very high percentage losses in the form of inedible products of many ecological "links."

Presently, the chemical synthesis of food, is restricted to a few simple types of food found in nature. Fortunately, however, the body requires a relatively small amount of the more complicated nutrients, and there is reason to believe that 80 to 90 percent of the caloric requirements could be satisfied by simple organic compounds that could be synthesized in space more economically than most, if not all, biologically produced nutrients. The hypothesis underlying this portion of the study was that it was preferable to synthesize the major portion of the diet chemically and carry the remainder as stored food rather than to synthesize a complete diet biologically. Obviously, there is some mission duration beyond which any system that requires no stored food is preferable to any system that requires stored food; but this study indicates, as have others, that such a duration may be well beyond the three years arbitrarily assumed to be the maximum duration of interest.

The problems to be solved in achieving a chemical solution are basically nutritional, chemical, and psychological and are central not only for space-flight but, perhaps, for feeding the rapidly expanding human race.

The following discussion is divided into four parts. Part 1 discusses the nutritional problems associated with formula diets. Part 2 discusses the synthesis of formaldehyde from CO_2 and H_2O . Part 3 discusses the synthesis of acceptable nutrients, mainly from formaldehyde. Part 4 analyzes the three selected processes for the manufacture of glycerol, fructose, and ethanol.

Nutritional Requirements

Basic nutritional requirements. - Many subtle problems concerning the use of formula diets in space remain to be solved. Total caloric and water requirements, vitamin, mineral, and other trace requirements, and effects upon intestinal flora are potentially important, but are not discussed in this report since the main conclusions are independent of these matters. For the purpose of comparing systems, a total energy requirement of 2800 kcal/day is assumed. Vitamin, mineral, and other trace requirements were assumed to be 30 g/day.

A widely quoted figure (for earth) for protein requirements is 0.5 g/kg of body weight per day, or 35 g for a 70-kg man. This assumes a high quality complete protein. The value is, an average for adults and is subject to moderate fluctuations among subjects. Nutritional guides for the public usually recommend twice this value for safety but the carefully controlled conditions of space diets should make the lower value a reasonable assumption.

Although the only identified human requirement for fat is that for about 2 g/day of unsaturated fatty acids, 20 g of fat per day is assumed.

Assuming a total energy requirement of 2800 kcal/day, and assuming 35 g of protein yield 140 kcal and 20 g of fat yield 180 kcal, 2480 kcal remain to be supplied by carbohydrates. All of which is to say that, assuming that the carbohydrate is nutritionally and psychologically acceptable, almost all of the carbohydrate that could be synthesized from the daily production of CO₂ and water could be utilized as food. Even if all of the protein and fat portions of the diet were stored, this would reduce stored food requirements from, for example, 650 to 55 g/day, a saving of over 90 percent. Protein synthesis by chemical means might be possible, but the problems here are greater and the potential returns much less. Little effort was spent on fat synthesis during the study. The study of the chemical synthesis of food was limited to the study of the synthesis of ethanol, glycerol, and other three-carbon compounds that are known intermediates in metabolism, and the hexose, fructose.

Glycerol.- Glycerol seems as likely to be an acceptable major calorie source as any three-carbon compound and, tentatively, appears to be the easiest to manufacture from the wastes available. The fats normally consumed by man are polyesters of glycerol. The amount of glycerol from this source normally amounts to only a few grams per day. Successful human feeding experiments involving the ingestion of as much as 110 g of pure glycerol per day (in three doses) for 50 days are reported. Various adverse effects have been reported following the ingestion of larger amounts of glycerol at one time by man or animal. The adverse effects of glycerol recorded appear to be related to its osmotic properties and that the renal threshold is exceeded and lost into the urine when blood levels are elevated. On the basis of present information, the prediction is that glycerol can be safely used to supply 900 to 1400 kcal per day, but probably not more.

Ethanol.- The only obvious problem with ethanol is control of the rate of administration to prevent intoxication, nausea, and acidosis. When ethanol ceases to be a food and becomes a pharmacologic agent depends upon the blood level attained, that is, upon the rates of administration and absorption versus the rate of utilization. Reported rates of utilization indicate that man could ingest from 7 to 17 g/hr without pharmacologic effects. This amount might provide from 50 to 75 percent of all calorie requirements if the ethanol were ingested in small amounts every one to two hours.

Many detrimental effects have been linked with ethanol, but most, if not all, appear to be explainable either as a result of drinking to the point of intoxication, that is, beyond the rate described above as permissible, or as side effects from not eating required protein or other nutrients. It is necessary to supplement an ethanol diet with 90 to 120 g of protein and/or carbohydrate per day to prevent the accumulation of ketone bodies (ketosis), but such amounts would be provided anyway for other reasons. The psychological problem of controlling the rate of administration was considered basically to be beyond the scope of this study, but such a problem could be limiting. It was felt earlier during the study that there was an excellent chance that a mixed diet of, for example, 1/3 ethanol and 2/3 glycerol would be superior to either separately. Two reports were found later in the literature, however, that glycerol uptake is reduced to about 1/3 the control

value in the presence of ethanol. This phenomenon is thought to be associated with accumulation of reduced diphosphopyridine nucleotide in the liver during ethanol metabolism. An ethanol-fructose combination apparently would not present this problem.

Fructose. - Fructose occurs with more or less glucose in plant juices, in fruits, and especially in honey, of which it constitutes about one-half the solid matter. Fructose serves, like glucose, for the production of glycogen.

Because none of the literature reviewed mentions which optical isomer of fructose was used in feeding studies, it is assumed that D-fructose was used. With moderate test dosages, fructose is as completely absorbed but at 65 per cent of the rate of D-glucose. Its rate of metabolism is not depressed in stress situations (unlike that of glucose), and it appears to be metabolized more than twice as rapidly as glucose. Fructose has the interesting effect of almost doubling the rate of metabolism of ethanol. Successful human feeding studies are reported for ingestion rates up to 250 g/day. Higher rates are not reported.

It has been reported that serum cholesterol values are higher when sucrose is present in the diet than when glucose is given. Since sucrose is not absorbed as such, this effect is probably due to the fructose moiety, but no specific evidence on this point was found. Except for these considerations and that fructose is extremely sweet and the general problem of osmotic load, there seems to be no bar to its utilization as a major energy source.

D- vs. L-carbohydrates. - Since the resolution of a racemic mixture is the most difficult step in the synthesis of a D-carbohydrate, for example, D-fructose, an attempt was made to verify the necessity of feeding D-carbohydrates. A reasonable search has not yielded any information on the peroral administration of L- or racemic carbohydrates. In vitro, L-sorbose is a strong inhibitor of hexokinase and DL-glyceraldehyde inhibits glycolysis. DL-glyceraldehyde is five times as effective as D-glyceraldehyde in inhibition of incorporation of glycine into mouse ascites tumor-cell proteins; both inhibit respiration and tumor growth. It has been reported that DL-allothreonine forms glycogen in the rat quite as effectively as does DL-threonine, although only D-threonine fulfills the essential amino acid role. This suggests, but does not prove, that L-threose may be utilized and is probably harmless at low dosages. Evidence indicates that L-isomers and racemic mixtures should be avoided, but this problem deserves further study.

Formaldehyde Synthesis

Early in the study it was concluded that based on analytical chemical considerations supported by a literature survey, edible polycarbon compounds are most economically formed using formaldehyde (HCHO) as an intermediate. Russian workers have considered the food synthesis problem and have also concluded that formaldehyde synthesis as an intermediate step is more feasible than direct carbohydrate synthesis from CO and H₂. This section considers several methods of producing HCHO from CO₂ and H₂O. Many of the concepts

assume CO and H₂ to be the available reactants. No discussion is presented in such cases of methods of producing CO from CO₂ or H₂ from H₂O since such methods are being developed in connection with other aspects of spacecraft life support systems.

A literature search revealed very little recent or current work pertinent to formaldehyde synthesis. In a NASA-funded study, HCHO synthesis was attempted in a silent electrical discharge by the direct reaction:



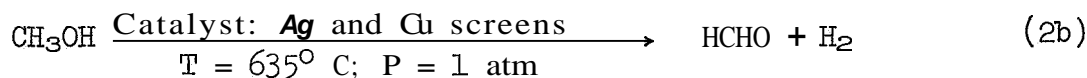
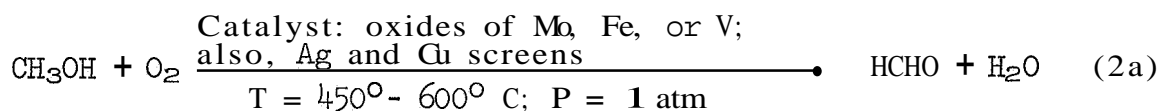
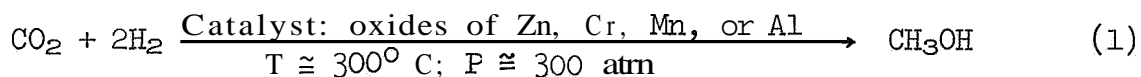
Maximum yields were found to occur with the stoichiometric reactant ratio; however, these yields were less than 1 percent.

Van Rysselberghe, using quaternary ammonium and potassium salts as electrolytes, found that electrochemical reduction of CO₂ to formic acid (HCOOH) took place with 100 percent current efficiencies on mercury cathodes, apparently at ambient temperature and pressure. He has not noted formaldehyde synthesis by this process.

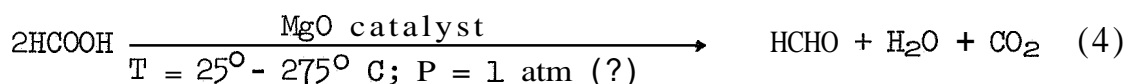
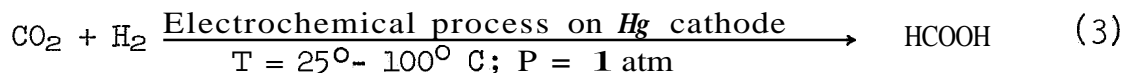
Other NASA-funded work in 1962 centered about electrochemical studies on the cathodic reduction of CO₂. Only qualitative data was reported, and apparently only trace quantities of formaldehyde were detected. The reduction was accomplished "at extremely low efficiencies."

Two other reaction schemes are potentially suitable for formaldehyde synthesis in a space vehicle and are designated as process A and process B. These processes can be represented as follows:

Process A



Process B



All the steps of process A are currently performed routinely in industry, and have been proven to be intrinsically sound. This process does, however, involve a high pressure step, equation (1).

Process B is recommended for further study; feasibility remains to be proven for the second step. As mentioned above, the step indicated by equation (3) has been previously reported with high yields. The decomposition of HCOOH to HCHO in a static system has been recently noted by Dutch investigators by infrared spectra at absolute pressures of a few torr. The present status of process B must be considered that of a laboratory curiosity. Quantitative data and other information needed for reactor design are not available. Nonetheless, this process is potentially of great interest because it provides an ambient pressure and temperature route to formaldehyde synthesis.

Process A, methanol synthesis, equation (1).- Theoretically, CO and H can react to form a large number of products. Thermodynamic information and some of these reactions are shown in table VI.

TABLE VI.- TYPICAL COMPETING REACTIONS IN METHANOL SYNTHESIS FROM
CO AND H₂ REACTION

		$\Delta H^{\circ}_{25^{\circ}C}$	$\Delta F^{\circ}_{25^{\circ}C}$	$\Delta H^{\circ}_{327^{\circ}C}$	$\Delta F^{\circ}_{327^{\circ}C}$
(5.1)	$CO + 3H_2 = CH_4 + H_2O(g)$	-49.3	-34	-52	-17
(5.2)	$2CO + 5H_2 = C_2H_6 + 2H_2O(g)$	-83	-52	-88	-18
(5.3)	$3CO + 7H_2 + C_3H_8 = 3H_2O(g)$	-119	-71	-125	-21
(5.4)	$CO + 2H_2 = CH_3OH(g)$	-22	-6	-24	11
(5.5)	$2CO + 4H_2 = CH_3OCH_3(g) + H_2O(g)$	-50	-16	-53	19
(5.6)	$2CO + 4H_2 = C_2H_5OH(g) + H_2O(g)$	-61	-29	-65	4.5
(5.7)	$3CO + 6H_2 = C_3H_7OH(g) + 2H_2O(g)$	-99	-53	-104	-4.4
(5.8)	$2CO + 2H_2 = CH_4 + CO_2$	-59	-41	-61	-21.2
(5.9)	$3CO + 3H_2 = C_2H_5OH(g) + CO_2$	-71	-36	-74	-0.57
(5.10)	$2CO = C(s) + CO_2$	-41	-29	-41	-16

Formation of paraffin hydrocarbons (reactions 5.1 - 5.3 and 5.8) is favored thermodynamically over the methanol reaction at all temperatures; high pressures have the greatest favorable effect on reactions 5.4 to 5.7 and 5.9 because of a 3:1 volume contraction with reaction. The keys to high methanol yields are catalyst selectivity, appropriate choice of temperature and pressure, and high space velocity (20,000 to 50,000 hr⁻¹).

Because of the heat release from the reaction of 24 kcal/g-mole methanol, no heat needs to be added to the reactor once the reaction has begun to maintain the temperature at 300° C. Temperature is kept constant by maintaining proper space velocity and heat exchange. Reactant gases are compressed to 300 atm and heated in counter-current exchangers by the product gas. Product gases are expanded to 1 atm pressure and cool liquid methanol of 99 percent purity is decanted. Conversion per pass of CO is about 15 percent.

Process A, methanol reaction to formaldehyde, equations (2a) and (2b).-

A mixture of methanol vapor and air or oxygen is passed over a fixed catalyst bed at atmospheric pressure. The product gases are absorbed in water and then separated by distillation. When oxides of Mo, Fe, or V are used as catalysts, the mechanism is believed to be that represented by equation (2a). Silver and copper metal catalysts appear to result in both oxidation and dehydrogenation, represented by both equations (2a) and (2b). Water vapor has a favorable effect on the metal-catalyzed process in that the reaction temperature is lowered and side reactions are inhibited. In the metal-catalyzed process, the feed is about 50 volume percent CH_3OH and 50 volume percent air, whereas 5 to 10 times as much air is required by the oxide-catalyzed process. One reason that less diluent gas is required for cooling by the former process is that it is a combination of an exothermic (oxidation) and an endothermic (dehydrogenation) reaction, so that the heat from one reaction tends to be consumed by the other.

The hot product gases from the catalytic reactor are delivered to a column where a preliminary separation of HCHO and unreacted CH_3OH occurs by scrubbing and cooling. The gases then pass to a final scrubber fed with cold water and are then vented. For the case of an air feed and a metal catalyst, the exit gases contain about 20 percent hydrogen. For 75 percent nitrogen, 5 percent CO_2 , and traces of CO, oxygen, and methane which represent direct loss of methanol by over-oxidation. The combined condensate and scrubber liquid from the main column (containing HCHO, water, and about 15 percent methanol) are fed at suitable points into a fractionating column, where 37 percent formaldehyde plus the desired amount of methanol stabilizer is removed from the bottom, and excess methanol is removed from the top and returned to the feed vaporizer. Overall net yields of 85 to 90 percent by weight based on the methanol fed are obtained by using either the metal or metal oxide catalyst.

A priori, there is no apparent reason why the current commercial processes for formaldehyde synthesis from CO and hydrogen as represented by process A could not be used for space applications, provided that the operation of integral units such as distillation and scrubbing columns can be carried out under zero g and other spacecraft conditions. The steps involved should be investigated under laboratory conditions with a view toward: (1) developing reaction and separation units that are more compact and lighter than presently available commercial units, and (2) performing a catalyst study to optimize catalyst life and poisoning resistance. Certain precious-metal catalysts may offer substantial advantages but have not been extensively studied for commercial use due to cost limitations.

A laboratory study should be made to determine if process B can be used on a continuous basis with reasonable yields. Process B is intrinsically more attractive because no high pressure steps are involved. Process B, however, may involve a gas buildup at the electrode under zero g.

The Synthesis of Edible Polycarbon Compounds

In this portion of the study, the main emphasis was on the synthesis of glycerol, D-fructose, and ethanol. The first two syntheses were assumed to use formaldehyde as a starting material. The syntheses of polypeptides and certain other compounds are discussed briefly.

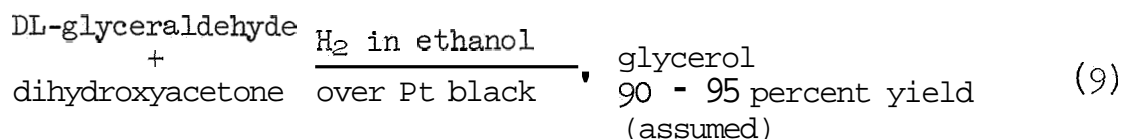
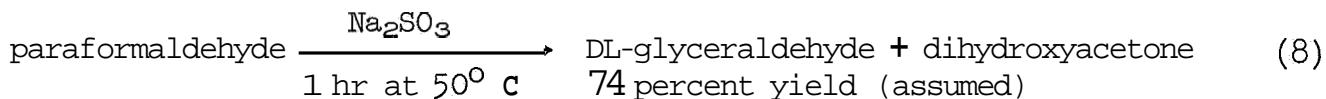
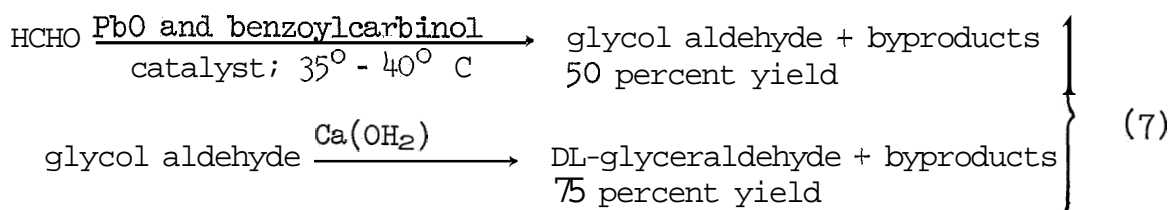
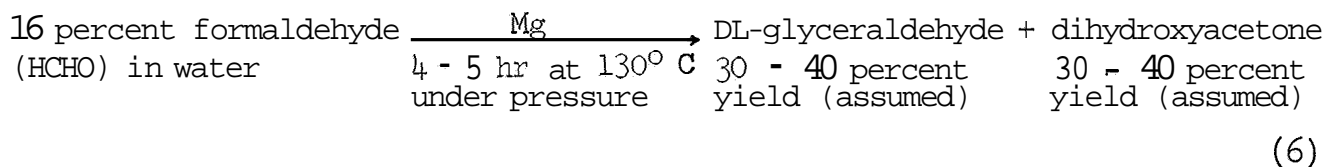
The chemical synthesis of proteinoids.- Several syntheses of amino acids from simple gases have been reported. Two of the earliest methods used an electric discharge in gaseous mixtures of methane, hydrogen, water, and ammonia. Other sources of energy used for similar syntheses were ultraviolet light and α , β , γ , and X-rays. A novel approach to the problem involved the use of thermal energy. The last method involved vapor phase thermal reactions of methane, ammonia, and water. Typical results yielded racemic mixtures of amino acids and small (2 - 8 units) polypeptides. From the 18 nonsulfur-containing amino acids common to proteins, 14 were produced simultaneously. It is significant that these 14 amino acids are common to protein and that no other amino acids were found. The above experiment indicated successfully that simple gases are thermally convertible to most amino acids. This approach appears promising for studies directed toward prolonged missions. However, considerably more research will be needed to determine all the variables and develop good yields. The sulfur-containing amino acids are not synthesized in the above-mentioned process. Further work in this area is not recommended for the immediate future.

High energy nonfat nutrient sources.- In an attempt to discover high energy nutritious compounds, certain organic molecules not found in naturally occurring foods have been studied. Among the compounds examined, 1, 3-butanediol and 2, 4-dimethylheptanoic acid were promising. It has not yet been proven that such compounds are acceptable human dietary nutrient sources. While these two compounds are not considered feasible candidates for space-craft synthesis, the considerable successful animal nutrition work with the compounds indicates the general versatility of mammals with respect to acceptance of unusual energy sources, and particularly their ability to utilize small molecular weight compounds.

The catalytic condensation of formaldehyde to glycerol and to fructose.- For many years it has been known that formaldehyde is condensed in the presence of base to yield a sweet syrup consisting of different sugars and other organic compounds. Feeding experiments using such syrups with rats, however, were not successful. Paper chromatography indicates that the sweet reaction product typically obtained from such a formaldehyde condensation consisted of many organic compounds among which about six hexoses, four pentoses, as well as erythrose and threose, were present. Each of these sugars is in the DL form since the reaction conditions are nonstereospecific.

Direct utilization of such nonspecific condensations was not feasible because of the low yields of useful products, complicated separation techniques, and difficult resolutions required. Instead, an intensive search was made of specific catalysts and reaction conditions that would give high yields of desired products. The most promising methods identified are summarized below.

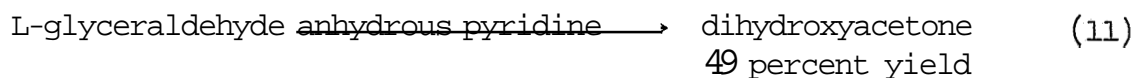
Glycerol.- Three promising methods for the production of a mixture of DL-glyceraldehyde and dihydroxyacetone from formaldehyde were found. These reactions are represented by equations (6), (7), and (8). Irrespective of the methods used, the following step is the same, that is, the hydrogenation of the triose mixture over a platinum black catalyst as shown by equation (9).

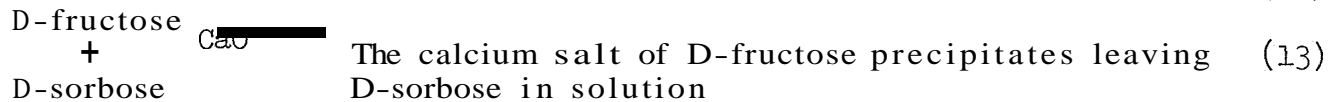
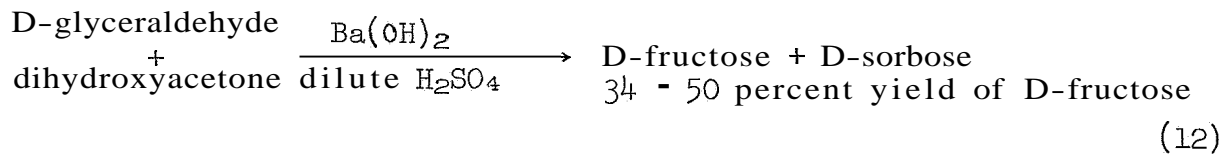


D-fructose.- The synthesis of D-fructose also starts with one of the three reactions shown in equations (6), (7), and (8). Two possible routes can be used from there to D-fructose. The first route involves the resolution of DL-glyceraldehyde and the synthesis of D-fructose by the condensation of D-glyceraldehyde and dihydroxyacetone. The second route involves the synthesis of DL-fructose from DL-glyceraldehyde and dihydroxyacetone and the subsequent resolution of the DL-fructose. The choice would be determined by the relative ease of the two resolutions. Only the first route is shown below (eqs. (10), (11), (12), and (13)); but a more thorough investigation might show resolution of D-fructose to be the easier.



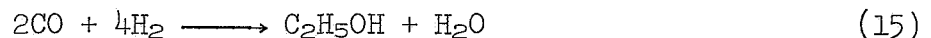
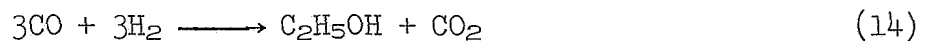
(This step requires considerable research. Only tedious methods for this step are documented. It is suggested that resolution be tried by column chromatography with an optically active substrate.)





The D-fructose is recovered by passing a stream of CO_2 through a water suspension of the calcium salt. The calcium carbonate is then removed by filtration.

Ethanol synthesis.- The two most promising routes to ethanol are:



Equilibrium data indicates that reaction (14) is favored by an order of magnitude over reaction (15) and since kinetic data were unavailable, reaction (14) is used as the basis of plant design. Reaction conditions are 190°C and 30 atm pressure. An 80 percent yield of alcohols, of which 12 percent is ethanol, is reported. A space velocity of 100 to 500 hr^{-1} is used and the catalyst is similar to standard Ruhrchemie iron catalyst, which contains copper, calcium oxide, and diatomite impregnated with KOH. The problems with the ethanol process are mainly the same ones common to the glycerol and fructose processes (i.e., finding the right catalyst and separating the desired reactants from a mixture and high recycle requirements resulting from low yields). The ethanol process originally seemed simpler than the glycerol process because it was basically a single step reaction. Close analysis, however, revealed that the higher recycle rate, long residence time, and low yields made the ethanol system considerably heavier than the glycerol system. This comparison is somewhat unfair to the ethanol system in that commercial ethanol figures were compared with laboratory glycerol yields. There is some reason to hope that the ethanol yields could be increased substantially by the use of more expensive catalysts.

Process Analysis

Estimates of weight, power, and thermal requirements for the synthesis of glycerol, D-fructose, and ethanol were made, based on available data. These compounds were judged the most promising of all those considered as a major energy source. Starting materials for syntheses are assumed to be CO and H_2 . To close the ecological system, all chemical plant wastes are incinerated to CO_2 and H_2O , and the products electrolyzed to regenerate starting materials. Synthesis plants are charged the equivalent penalty for this electrolysis, which is an additional load on the environmental control system

electrolysis units. The inevitable small loss of carbon in the synthesis cycle is accounted for as free carbon production, and amounts to a few hundredths of a pound per day.

Tables VII, VIII, and IX show the estimates made, and figures 2, 3, and 4 show the process flow charts for the three syntheses - glycerol, fructose, and ethanol.

TABU VII.- GLYCEROL SYNTHESIS PLANT ESTIMATES (10 MEN)

Step	Weight, lb	Electrical power, kW	Thermal requirements, kW	
			Heating	Cooling
Methanol (22.3 lb/day)	230	5.0	0.16 (570° F)	3.5
Formaldehyde (18.2 lb/day)	50	.002	.23 (1200° F) .36 (200° F)	.97
Trioses (13.4 lb/day)	20	.002	.08 (200° F)	.08
Glycerol (13 lb/day)	160	.002	6.7 (200° F)	6.7
Waste incinerator (9.3 lb/day)	25	---	neg .	.6
Total	485	5.0	.23 (1200° F) .16 (570° F) 7.14 (200° F)	11.85

TABLE VIII.- D-FRUCTOSE SYNTHESIS PLANT ESTIMATES

[Basis: 13.9 lb/day]

Weight, lb	Electrical power, kW	Thermal requirements, kW	
		Heating	Cooling
2700	22	3.3 (1900° F) 1.0 (1200° F) 6.9 (570° F) 17.0 (200° F)	41 (of this 0.22° to 35° F)

TABLE IX.- ETHANOL SYNTHESIS PLANT ESTIMATES

[Basis: 3.95 lb/day]

Weight, lb	Electrical power, kW	Thermal requirements, kW	
		Heating	Cooling
900	0.45	1.4 (200° F)	6.2

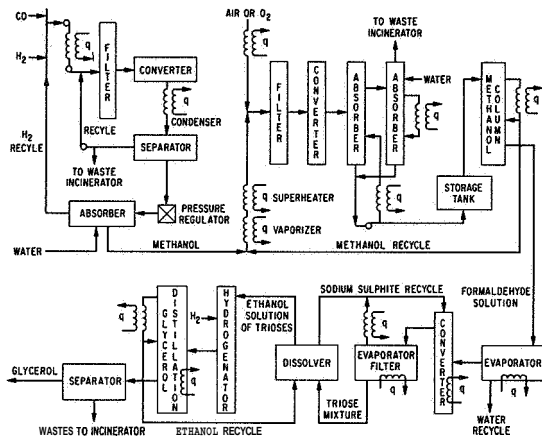


Figure 2.- Glycerol synthesis flow chart.

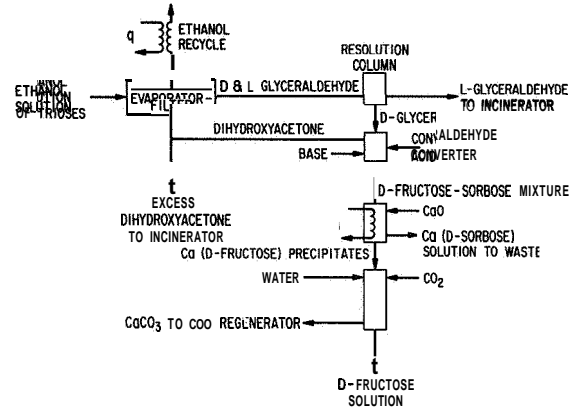


Figure 3.- D-fructose synthesis flow chart.

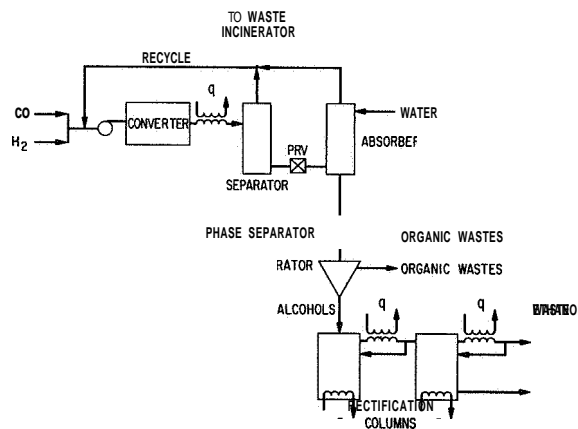


Figure 4.- Ethanol synthesis flow chart.

BIOLOGICAL SYSTEMS

Since space travel was first discussed, biological systems, chiefly photosynthetic, have been suggested as ways of closing the spacecraft ecological cycle. Elaborate systems have been conjured up, including the use of fish, chicken, and cattle that subsisted on the plants and provided man his preferred foods. For every pound of steak, butter, eggs, or strawberries

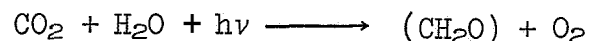
produced, however, several pounds of inedible horns, skin, bones, stems, roots, and other materials are also produced. The latter could only be recycled with great difficulty by incineration or microbial digestion.

The calculations made during the study indicate that a biological system that is economical for one-to three-year missions must be largely edible as grown and must lend itself to extended reliable automated growth and harvesting. Calculations also indicate that no biological system ever investigated seems economical as a gas exchange medium alone, but must be used as a major food source if it is to find application. The power penalty is an important factor. The precise influence of this variable is indicated in the following section, but it might be generally noted that the economy of biological systems for space flight is highly dependent upon the development of efficient, reliable nuclear or solar power systems that produce power for a few hundred pounds per kilowatt or less.

The following section is divided into four parts. The first part deals with the three types of biological systems that have received considerable study and seem most promising, namely, algae, Hydrogenomonas, and duckweed. The second deals with little studied or unstudied biological systems; the third part contains the results of an evaluation of a system that would utilize natural light for photosynthesis, and the fourth section contains system analyses of the three most promising systems.

Candidate Concepts

Two types of biochemical reactions are being considered in the development of a biological regenerative life support system. The first type of biochemical reaction is photosynthesis. Photosynthesis is the complex process by which cell materials are synthesized from CO_2 and H_2O by living plant cells, using visible light as an energy source. Photosynthesis can be represented by the following generalized equation:



Human metabolism, on the other hand, involves the oxidation of cell material with atmospheric oxygen to obtain needed energy and the accompanying release of CO_2 and H_2O as waste products.

The second biochemical reaction, which is much less familiar, combines the electrolysis of H_2O to produce oxygen with the chemosynthetic reduction of carbon dioxide by hydrogen-fixing bacteria:



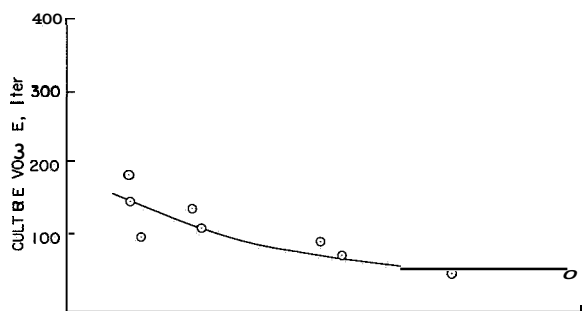
First hydrogen and oxygen are combined to obtain energy; then CO_2 is reduced to form cell material. Both the photosynthetic and the chemosynthetic systems require energy inputs to reduce CO_2 . In the photosynthetic system, the pigment chlorophyll a traps visible light energy to carry out the reduction; energy is obtained by the oxidation of hydrogen in the chemosynthetic

one. In either system the organism is a potential food source for man and also a potential waste disposal system for urine and feces.

Phyotosynthetic organisms under study are algae and several types of vascular plants. The former include Chlorella, Synechococcus, Scenedesmus, and Anacystis and the latter, duckweed, lettuce, endive, Chinese cabbage, and sugar cane. Plant tissue cultures have also received attention. Research on chemosynthetic organisms has dealt mainly with species of Hydrogenomonas, the hydrogen-fixing bacteria.

The present bioregenerative life support systems review consolidates and compares the information pertaining to gas exchange, food production, and waste removal for each organism studied. Published research on biological regenerative life support systems served as a basis for selecting preferred biological systems.

Algae.- A great deal of research has been concerned with the weight, volume, and power requirements of an algal photosynthetic gas exchanger. Most work has been done with Chlorella pyrenoidosa or Chlorella TX71105, a high temperature strain. C. H. Ward's comparison of such work included estimates of culture volume varying from 3 to 3000 liters per man and estimates of required power ranging from 7 to 100 kW per man. Comparison of these data is difficult because many key parameters were unrecorded, for example, culture density, culture thickness, light intensity, spectral energy distribution, CO₂ concentration, algae species, nutrient media, pH, and temperature. After a careful screening, nine of the most optimum working photosynthetic gas exchangers were selected and their data are plotted in figure 5.



Data is restricted to Chlorella, with one exception. Culture volume and input power are inversely related, as would be expected. Higher levels of energy are less readily absorbed, and, even if absorbed, cannot be used for photosynthesis because of the rate-limiting effect of enzymatic reactions.

Continuously growing cultures of Chlorella pyrenoidosa have produced uniform photosynthetic quotients ($PQ = \frac{CO_2}{O_2}$) for as long as 180 days.

Contamination of an algal culture with bacteria or other organisms has been shown to adversely affect the productivity and reliability of an algal gas exchanger. Ward et al. found that the presence of the bacteria, Mima polymorpha and Pseudomonas aeruginosa, caused a 20 percent decrease in algal growth. Blasco noted a slight inhibitory effect on algal growth in two or three bacterial isolates. Bacteria have been associated with large changes in pH in the culture media and the clumping of algae cells. The latter phenomenon rapidly reduces O₂ production. Satisfactory reliability thus far appears to be associated only with pure algal cultures.

Studies to date have not achieved a balanced gas exchange between algae and man (or animal). This is not surprising, however, since no experiment is recorded in which any attempt was made to control the human (or animal) diet. The RQ's reported for algae range from 0.87 to 0.95; this is somewhat surprising since a diet of typical algae would be expected to produce a human RQ of 0.82 to 0.85. This discrepancy should become minimal, however, in a completely closed system in which algae and man provided nearly 100 percent of each other's nutrients and cellulose was recycled by incineration.

The ability of algae to serve as a food source has been assessed from two viewpoints: (1) composition of algae under a variety of environmental conditions and (2) feeding studies. Chemical analyses indicate that algae, with minimal supplementation, should potentially be an excellent food. *Chlorella pyrenoidosa*, as ordinarily grown, has an apparent composition of 40 to 60 percent protein, 10 to 20 percent fat, 5 to 10 percent ash, and approximately 20 percent carbohydrate on a dry weight basis. The level of "essential" amino acids appears adequate with the exception of the sulfur-containing amino acids, methionine and cystine. Vitamin and mineral supplements required are not significant from a mass viewpoint.

Few feeding studies are reported in which algae was as much as 20 percent of the diet. A comprehensive large-scale human feeding experiment was carried out by Powell, Nevels, and McDowell. All subjects tolerated diets supplemented with algae in amounts up to 100 g/man/day. Some gastrointestinal symptoms, consisting of abdominal distension, increased eructations, and flatulence appeared. The dominant taste was similar to bitter spinach or strong green tea, although subjects became accustomed to the flavor. At feeding levels above 100 g/day, these symptoms increased along with nausea, and in some subjects, diffuse abdominal pain, vomiting, malaise, and headache. Stools became bulky and dry. Only two of four subjects were able to ingest 500 g of algae as the only food source and not without symptoms. Physical examinations failed to show abnormalities other than those associated with the gastrointestinal tract.

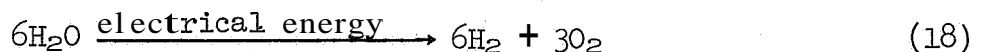
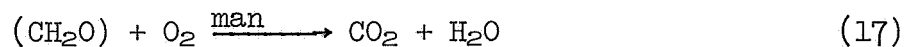
The gastrointestinal symptoms may be due to toxins produced by bacteria or algae. Extraction of carotenes, chlorophylls, and xanthophylls leaves a more digestible product. Poor digestibility of algae may be due to its tough cell wall. The cellular membrane often remains intact when dried algae is consumed and stools appear green in color. Attempts to increase digestibility of algae cells have led to enzyme studies to break the algal cell wall. Feeding amylase or pectinase increased digestibility. More studies on the

nutritive value of algae are required. Of first importance are studies that would lead to improved acceptability and digestibility when algae comprise a large percentage of man's diet.

Waste recovery processes coupled with an algal photosynthetic gas exchanger that have been studied include incineration, electrochemical degradation, aerobic digestion, anaerobic digestion, and direct algal utilization. All of these methods can be used successfully with algae, but incineration or aerobic digestion of solid wastes appears most desirable from engineering considerations. Direct utilization of urine is possible if periodic treatment of the nutrient fluid removes the buildup of trace substances not utilized, for example, allantoin, creatinine, and hippuric acid.

Hydrogenomonas. - A few years ago, it became obvious to many that the high power requirement of algae and other photosynthetic systems, or the alternative high weight of apparatus necessary to the use of natural light, was likely to limit the usefulness of such systems for space flight. An attempt was made to find an organism that more efficiently uses its power. As mentioned in the previous section, algae have a theoretical maximum efficiency of 20 percent relative to light absorbed, but the most efficient electric to light energy conversion currently available is also 20 percent so that 4 percent is the net maximum efficiency achievable. In practice, it is believed that about 2 percent efficiency is the best realistic estimate for the foreseeable future.

From a number of candidate bacteria, Hydrogenomonas was selected and has been studied under NASA auspices for the last three years in half a dozen laboratories. Hydrogenomonas reduces CO₂, producing cell material and H₂O; the energy for this reduction is obtained by the oxidation of hydrogen with molecular oxygen. Therefore, this biological system functions directly as a CO₂ reducer only. Oxygen for man and the bacterium must be obtained by another system, that is, electrolysis of water. An integrated system can be written as follows:



Equation (16) represents an idealized net reaction performed by Hydrogenomonas and shows the utilization of six hydrogen molecules and two oxygen molecules to reduce one molecule of CO₂ to cell material. Man's oxidation of food with oxygen to produce CO₂ is shown in equation (17). The input of energy to split water to produce the oxygen and hydrogen required in equations (16) and (17) is shown in equation (18). This balanced system is an idealized situation for a closed system using man and Hydrogenomonas.

It appears that the efficiency of the Hydrogenomonas is over 40 percent. Current predictions are that the electrolysis of water in space can be accomplished with about 75 percent efficiency for a net efficiency of over

30 percent or over 15 times the efficiency of photosynthetic systems. While this advantage is substantial, the results of this study indicate that the major savings are not directly related to the reduced power requirement of Hydrogenomonas, but are a result of the immensely greater ease of distributing the power. The high fixed weight of the algal system - that is, large culture volume, high weight of containers due to the necessity of achieving a large surface area, and high weight of lights and associated apparatus - are all due to the difficulty of distributing the energy to the algae. Energy distribution, on the other hand, is a simple matter with Hydrogenomonas. Bongers has estimated from the dry weight of cells produced during a 4-hour period that, for a Hydrogenomonas system, 10 to 15 liters of culture are required to reduce the CO₂ produced by one man, and 1 kW of electricity is required for the electrolysis of water to produce the oxygen required by one man and the hydrogen and oxygen required by the Hydrogenomonas system to reduce the CO₂ produced by one man.

Hydrogenomonas has not as yet been grown on incinerated, electrochemical, anaerobic or aerobic digester products. The organism does utilize urea as a sole nitrogen source. It also grows on 5 to 100 percent urine; gas uptake is best with 100 percent urine. Much of the information on the buildup of waste removal products presented for an algal system applies to Hydrogenomonas. The electrochemical waste removal system may be advantageous for this organism. During electrolysis, some water is split into hydrogen and oxygen, which are the gases needed to reduce CO₂.

Organisms harvested from a continuous culture containing an adequate nitrogen source were found to be composed largely of protein with a small amount of lipid and very little carbohydrate. Hydrogenomonas grown on ammonium chloride contained 11.4 percent total nitrogen while cells grown on urea contained 13.5 percent total nitrogen. These values are equivalent to approximately 70 to 80 percent protein. Lipid values from 0.41 percent to 9.37 percent have been obtained depending on the extraction method. The content of individual amino acids reported by Foster and Litchfield appears to be representative of other microorganisms, for example, unicellular algae. Most of the essential amino acids are present with the exception of those containing sulfur.

The composition of Hydrogenomonas is known to vary greatly depending on the environmental conditions. Low concentrations of oxygen (5 - 10 percent) favor gas uptake, but cell metabolism leads to formation of a storage compound, poly-β-hydroxybutyric acid. An inadequate nitrogen supply can also lead to an accumulation of this lipid-like polymer. The nutritional and toxic properties of this compound are not known.

Very little is known about the nutritional value of Hydrogenomonas. Dr. Doris Calloway at the University of California in Berkeley has worked and is currently working on the biological value of the bacterium. She has fed Hydrogenomonas as a sole source of protein to weanling rats. Boiled cells yielded 93 percent nitrogen absorption and sonicated cells 94 percent compared to a 99 percent casein control. Thus, the development of processing

procedures to make cell constituents available for food does not appear necessary. The protein quality seemed comparable to that of the casein control.

Few serious problems have been identified with the use of a Hydrogenomonas life support system for space flight, but the limited amount of work accomplished in this area leaves numerous problems unsettled. Feeding studies have only just begun; long-term reliability is a complete question mark. It is encouraging, however, that Hydrogenomonas systems appear quite profitable for one-year missions if even 20 percent of the cell material can be used as food, whereas algal systems appear useful for two-year missions only if almost 100 percent of the cell material is consumed as food.

Vascular plants. - Higher green plants possessing leaves, stems, and roots have been considered as components of a photosynthetic gas exchange system. Their primary advantage might be in ease of gas exchange. These plants are aerial in growth habit with their leaves (site of the photosynthetic apparatus) in direct contact with their gas environment. Use of vascular plants would eliminate the problem of obtaining adequate gas exchange between a gas and a liquid interphase as is necessary in the two other general systems under consideration (photosynthetic-algal and chemosynthetic-Hydrogenomonas).

The greatest disadvantage of these systems is the problem of automation. Automation of all the vascular plant systems would be difficult if not impossible and would, therefore, require considerable man hours for operation and maintenance.

Moss investigated the oxygen production of sugar cane leaves. The difficulty of illuminating all surfaces of the leaves of a growing plant greatly decrease the yield of the system. The cabbage, endive, and tampala systems investigated by Boeing Co. (1962) would experience the same problems. Tissue cultures of photosynthetic tissues, rose stem, tomato stem, and bracken gametophyte require a complex organic medium and sterile conditions.

The best representative of the vascular plants for a photosynthetic gas exchanger are members of the Lemnaceae. These are greatly simplified in structure with roots projecting directly from the leaf. Members of the Spirodela, Lemna, and Wolffia have been studied.

The leaves of Spirodela spread on the liquid surface by surface tension. Their upper surface is in direct contact with the gas phase. If proper circulation is maintained in the system, a CO₂ partial pressure of 0.2 mm Hg should be sufficient to maintain optimum growth. Most studies report a concentration of 1 to 5 percent CO₂ in the environment. The duckweed system eliminates the problem of a gas-liquid interphase exchange for both CO₂ and oxygen,

In a weightless environment, supply of liquid nutrients to the duckweed could be a major design and maintenance problem. Use of a solid inert matrix,

such as filter paper or vermiculite, would solve free water problems, but would create problems in harvesting and accumulation of undesirable products in the solid material.

Wilks estimates that a one-man duckweed system would involve 75 to 100 liters of culture medium, a volume of 25 to 50 ft³, and 25 kW of power.

Very little work has been done on the waste removal capabilities of duckweed or other vascular plants. Spirodela does grow with urea as a sole nitrogen source. It is expected that the same problems mentioned for algal waste removal will be encountered in duckweed.

Chemical analyses of Spirodela polynhiza cells indicate the possibility of feeding the organism as a sole source of nutrients in sustaining the life of an animal. Protein is 33.0 percent, fat 5.3 percent, fiber 4.9 percent, ash 13 percent, and carbohydrate 39 percent. Some vitamin, amino acid, and mineral determinations have also been carried out which indicate an adequate supply. Duckweed cells are easily ruptured. This should aid the human in digesting the cells. No experiments have been reported on the feeding of duckweed to humans. Adult mice fed duckweed (Wolffia) for 31 days survived with no weight loss. Four weanling rats fed a diet of 100 percent duckweed (Spirodela) died. Four adult mice fed Spirodela plus 20 percent paraffin for 31 days survived.

Generally, on the basis of the evidence at hand, no vascular plant system appears to be as reliable as an algal system and the lightest weight system appears to be at best as heavy as an algal system.

The Use of Natural Light for Algae Illumination

For many years the statement has appeared in the literature that the efficient utilization of natural light in space would make algal or other photosynthetic systems much more attractive. Because, at the beginning of this study, it was considered a possibly determining factor for biological system evaluation, the use of natural light for algae illumination was investigated in some detail. The results may be summarized briefly by saying that the use of natural light seems to reduce the power penalty for algal systems to about 200 lb per man. Photosynthetic systems studied were

so heavy otherwise that such a reduction in power penalty did not change the overall rating of the photosynthetic system relative to Hydrogenomonas or physicochemical systems. In addition, relatively few space missions include constant availability of natural light.

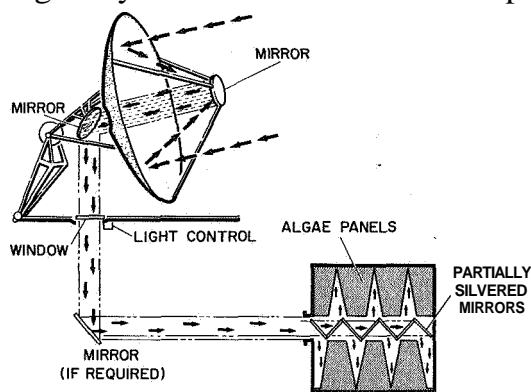


Figure 6.- A concept for the use of natural light for algal systems.

Figure 6 shows a typical concept for the use of natural light in space. A collector focuses light on a window, and

oriented, partially silvered mirrors distribute the light to algae beds. A more complicated system would be necessary for a rotating vehicle.

Mission	Artificial light	Natural light			
	Weight, lb (including power)	Direct window		Collector, 60:1 ratio	
		Weight, lb	Window area, ft ²	Weight, lb	Window area, ft ²
Earth, 10 men	4,540	2,500	260	1,055	800
Earth, 100 men	32,700	25,000	2,600	12,000	8,000
Mars, 10 men	4,540	7,500	650	1,700	2,000
Mars, 100 men	32,700	75,000	6,500	19,000	20,000

Columns 4 and 5 show the weights and collector areas required for the concept illustrated in figure 6, which requires only moderate orientation of the vehicle. The values shown assume a 60 to 1 concentration of energy by the collector upon a window. The latter system seems preferable since it is lighter and less restrictive upon vehicle design but suffers from the questionable reliability of the collector mechanism.

Values shown for Mars missions are based on the decreased solar flux at Mars. The collector concept suffers less than the window concept from this decrease in flux since a much smaller proportion of this system is affected by the size of the exterior flux.

The values shown for Earth missions are based on continuous availability of sunlight. This, of course, is not possible for any practical mission. Earth orbital missions would require about twice the values shown, assuming intermittent light would be acceptable. Lunar bases would also require about twice the values for Earth if 14 days storage of algae in the dormant stage and 14 days accumulation of urine, CO₂, and oxygen proved practicable. The most obvious application, that is, the Mars mission, involves the smallest, but still considerable, savings.

Less Studied Candidates for Microbial Life Support Systems

This portion of the study involved the examination of three CO₂-metabolizing bacteria; a dozen autotrophic bacteria capable of CO₂ fixation including aerobes and anaerobes, photosynthetic and nonphotosynthetic; several methane-oxidizing bacteria; one acetylene-utilizing bacterium; several yeasts and bacteria that utilize glycerol or fructose; and petroleum-metabolizing microbes.

While the results of this part of the study did not lead to any specific research and development recommendations, they did uncover some possibilities that were interesting enough to merit further analytical inquiry. It must be understood that a vast number of microorganisms can be identified that might be useful for spacecraft life support. It is of the nature of such microbial systems that the advantages or possible advantages are relatively apparent while the disadvantages generally require a great deal of painstaking research before becoming evident.

From this survey it is generally concluded that it would be desirable to study in more depth the microbiological utilization of glycerol, glyceraldehyde, and methane. Special consideration must be given to growth conditions, amount of growth, and generation times. The literature reviewed lacked information of this type. Consideration should also be given to the possibility of adaptation or mutation of an organism to the conversion of the substrate. Candidate organisms must be considered as possible food sources. At this time, a more comprehensive survey of microbes for space use is recommended before the start of any new research program.

Biological Systems Analysis

The state-of-the-art survey and subsequent study of biological systems resulted in the identification of the candidate approaches and the establishment of system requirements for the algal, Hydrogenomonas, and duckweed systems. Conceptual designs of these systems were accomplished to serve as a basis for weight, volume, and power estimates. This section presents the requirements, descriptions, and characteristics of the algal, Hydrogenomonas, and duckweed systems. Values for a 10-man, one-year mission only are given in this report.

Algae system. - The algal system requirements established during the state-of-the-art review are as follows:

Algae required as food	600 g dry wt/man-day
Algae culture volume	100 liters/man
Culture density	2.5 g dry wt/liter
Artificial lighting power	7 kW/man
Culture temperature	24° to 28°C
Culture thickness	1 cm (both sides lighted)
Culture growth rate	2.5 g dry wt/liter day
Harvesting	3 times/day
Culture bleed rate	10 liters/day

Subsystem tradeoffs were conducted to compare the algal pumping system and the Alгатron as gas exchange and light exposure concepts, and incineration and activated sludge as waste processing systems.

The algae pumping system was somewhat superior and is shown in figure 7 along with a system for processing algae for consumption. The algae pump circulates the algae culture through the gas exchange chamber and the light exposure panel. In the gas exchange chamber, the algae absorbs CO₂ from and releases oxygen to the cabin air. As the algae passes through the light chamber, energy is transmitted from fluorescent lights to the algae. As culture density increases to a prescribed level as a result of cell growth, the light intensity control energizes the harvesting valve and discharges algae culture to the harvesting tank. Cabin gas is circulated through the gas exchange chamber by a compressor.

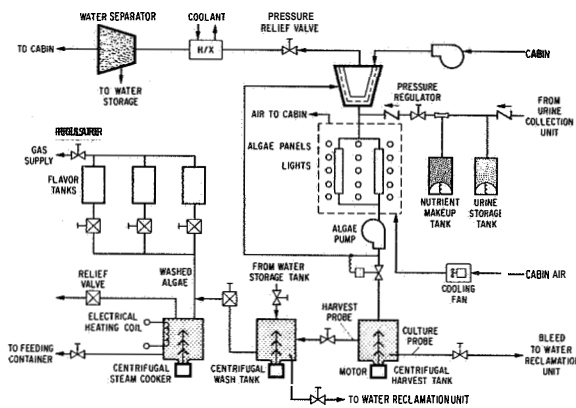


Figure 7.- Schematic diagram of algae system.

The oxygen rich, CO₂-free, saturated gaseous effluent of the gas exchanger passes through a heat exchanger and liquid-gas separator to remove excess moisture before returning to the cabin. The centrifugal harvesting chamber separates the algae cells and nutrient. The algae is transferred to the food processing unit by the harvest probe and the nutrient is returned to the algae circulation loop. A portion of the algae nutrient is bled to the water reclamation unit to remove contaminants that would otherwise build up in the circulation system. Makeup nutrient and urine are automatically introduced into the system to maintain the required nutrient composition.

Engineering calculations were based on the system requirements presented previously and the system schematic, figure 7. The algae-system-weight, volume, and power estimates are presented by table X. The components discussed above are listed as well as a water reclamation unit to process the constant culture bleed, a one-day urine storage tank, a heat exchanger and gas-liquid separator, a nutrient makeup supply, piping and controls, and the initial culture charge.

The incineration waste-handling system was selected because it is more predictable and it offers some weight, volume, and power savings. The problems of integrating these waste systems with a biological food-producing organism must be defined before a definite choice can be made.

Hydrogenomonas system.- The uses of Hydrogenomonas in a life support system were analyzed to establish the system requirements, the methods of meeting these requirements, and the weight, volume, and power characteristics of the system.

TABLE X.- ALGAL PUMPING-SYSTEM WEIGHT, VOLUME, AND POWER SUMMARY

[Ten-man, one-year mission]

<u>Subsystem</u>	<u>Weight, lb</u>	<u>Volume, ft³</u>	<u>Power, W</u>
Gas transfer	1660	191	2600
Cabin air compressor	100	1.0	1400
Gas exchange chamber	50	6.0	---
Light panel	1200	180.0	---
H-X and separator	50	.5	---
Cooling fan	60	1.0	1200
Spares	200	2.5	---
Algae circulation	3100	5.0	2700
Algae pump	200	2.0	2650
Water reclamation unit penalty	50	.2	50
Urine tank	30	1.0	---
Nutrient makeup	20	.2	---
Nutrient charge	2600	---	---
Spares	200	1.6	---
Lighting	1130	175.5	70,000
Light and ballast	800	170.0	70,000
Spare	330	5.5	---
Harvesting	250	2.5	700
Harvest tank	100	1.0	550
Piping and control	100	1.0	150
Spares	50	.5	---
Total	6140	374.0	76,000

System requirements.-

<u>Hydrogenomonas</u> required as food	586 g dry wt/man-day
Culture volume	10.6 liters/man
Culture density	10 g/liter
CO ₂ consumption	94 g/liter day
Gas consumption ratio	6H ₂ :2O ₂ :1CO ₂
Cell growth rate	23 percent/hr
Harvesting rate	3 times/day

The Hydrogenomonas system is shown schematically in figure 8. Because Hydrogenomonas consumes hydrogen, oxygen, and CO₂ and produces cell material and water, phase separation problems associated with gas exchange can be

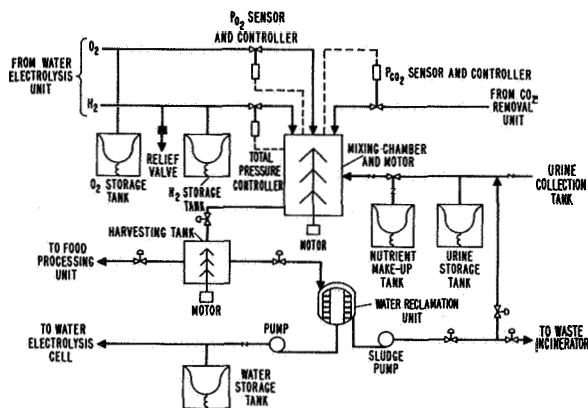


Figure 8.- Schematic diagram of Hydrogenomonas system.

eliminated. A closed culture chamber is used and hydrogen, oxygen, and CO₂ under pressure are supplied to the chamber. The flow of gases to the chamber is controlled by partial pressure sensors to maintain fixed H₂, O₂, and CO₂ concentrations in the chamber. The gases and culture medium are mixed by a paddle and baffle arrangement in the chamber to maximize gas diffusion into the liquid. The hydrogen and oxygen gases are supplied by a water electrolysis cell and the CO₂ is removed from the cabin air by a CO₂ removal device. The mixing chamber paddles are also used to deliver

Hydrogenomonas and nutrient to the harvest tank where centrifugal action is used to separate cell material from the nutrient. The nutrient from the harvest tank is processed in the water reclamation unit to remove any undesirable compounds which might otherwise build up in the culture chamber. The reclaimed water is used in the water electrolysis cell. The water reclamation unit waste water is delivered to an incineration unit. Nutrient makeup and urine are supplied to the mixing chamber during harvesting to maintain the required culture medium composition.

For the required 10.6 liters of culture medium per man, and allowing for the gas volume and chamber mixing blades and baffles, a growth chamber volume of 1 ft³ was used. The water produced by the Hydrogenomonas is 4.5 lb/man-day. The water reclamation unit was sized to handle this quantity of water. The harvest tank, urine tank, and the nutrient makeup tank were sized in the same manner as the algae system units. The Hydrogenomonas system weight, volume, and power for a 10-man, one-year mission are shown in table XI.

TABLE XI.- HYDROGENOMONAS SYSTEM WEIGHT, VOLUME, AND POWER SUMMARY
[Ten-man, one-year mission]

Equipment	Weight, lb	Volume, ft ³	Power, W
Mixing chamber with sensors, controls, and initial culture charge	770	11.0	850
Harvest tank	100	1.0	550
Urine tank	30	.6	---
Nutrient makeup tank	20	.2	---
Water reclamation unit	100	3.0	100
Water storage tank	30	.8	---
O ₂ surge tank	20	.4	---
H ₂ surge tank	30	.6	---
Piping and control	100	.4	150
Total	1200	18.0	1650

Duckweed system.- The characteristics of a duckweed life support system were established to compare this concept with other approaches to life support.

System requirements.- The following requirements were used to analyze the system.

Duckweed required as food	844 g dry wt/man-day
Culture light exposure area	120 ft ² /man
Lighting power input	7 kW/man
Culture temperature	75° to 85° F
Harvesting	Continuous

The duckweed system is shown schematically in figure 9. The culture panel is a hydrophilic sponge material reinforced with wire mesh stainless screen. The sponge material retains the liquid nutrient and the duckweed grows on both sides of the panel. The nutrient is pulled through the sponge by the nutrient pump. A pressure accumulator regulates the pressure on the sponges to keep them wet but not saturated to the point of breakthrough. An accumulator low-level switch activates the nutrient makeup valve to refill the accumulator. Fluorescent lights are equally spaced between the growth panels. A blower draws cabin air through the culture chamber to effect gas transfer and cooling of the panels. Water evaporated from the sponges is removed from the air in a heat exchanger/water separator unit prior to return to the cabin.

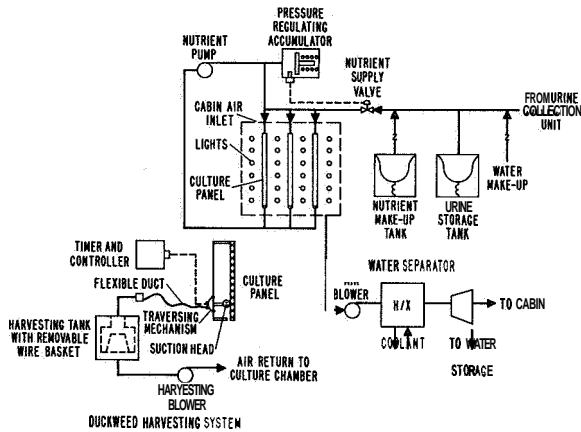


Figure 9.- Schematic diagram of duckweed system.

The duckweed harvesting system is designed around a harvesting blower that creates the suction required to remove the duckweed from the culture panels. A suction head picks up the duckweed and transports it by air to the harvesting tank. The air passes through the removable wire basket to the blower and returns to the culture chamber. The duckweed trapped in the wire basket is removed for processing. The suction head is programmed to automatically scan the culture panel in a programmed pattern to continuously harvest. Each element of the duckweed system was analyzed to provide the required systems data. The weight, volume, and power of a duckweed system for a 10-man, one-year mission is presented in table XII.

TABLE XII. - DUCKWEED SYSTEM WEIGHT, VOLUME, AND POWER SUMMARY

[Ten-man, one-year mission]

System	Weight, lb	Volume, ft ³	Power, W
Gas circulation	560	484	---
Culture chamber	345	480	---
Blower	55	1.2	800
H-X and separator	10	2.3	---
Control and ducting	20	1.0	60
Spare	30	.5	---
Nutrient supply	4070	20.0	100
Tank	30	.8	---
Makeup tank	20	.2	---
Culture panel	3700	17.0	---
Piping and control	120	1.0	---
Spare	200	1.0	100
Lighting with spare	930	2.0	70,000
Harvesting	310	8.0	1200
Suction head	25	.2	---
Tank	100	6.0	---
Blower	50	1.0	800
Timer and control	50	.2	400
Piping	30	.2	---
Spare	55	.4	---
Total	5870	514.0	72,160

CLOSED ECOLOGICAL SYSTEMS COMPARISON STUDIES

The physicochemical, biological, and chemical synthesis of food studies resulted in several potentially attractive approaches to life support in each category, which were analyzed in detail to establish subsystem characteristics. Schematics and weight, volume, and power data were generated for the subsystems. This section of the report compares these various approaches to life support.

IDENTIFICATION OF CLOSED ECOLOGICAL SYSTEMS

To compare the various candidate life support systems systematically, the various subsystems were combined into the following 14 total systems.

System No.	Identification
1	Physicochemical system A with stored food
2	Physicochemical system B with stored food
3	Physicochemical system B with glycerol
4	Physicochemical system B with glycerol-ethanol
5	Physicochemical system B with fructose
6	Physicochemical system B with fructose-ethanol
7	Algae - 100 percent for gas exchange; 100 percent food
8	Algae - 100 percent for gas exchange; 50 percent food, 50 percent stored food
9	Algae - 50 percent for food and gas exchange; 50 percent physicochemical system B with glycerol
10	Algae - 100 percent for gas exchange; 100 percent stored food
11	<u>Hydrogenomonas</u> - 100 percent for CO ₂ reduction; 100 percent food
12	<u>Hydrogenomonas</u> - 50 percent for CO ₂ reduction and food; 50 percent physicochemical system B with glycerol
13	<u>Hydrogenomonas</u> - 100 percent for CO ₂ reduction; 100 percent stored food
14	Duckweed - 100 percent for gas exchange; 100 percent food

Systems 1 and 2 are the selected physicochemical systems with the addition of stored food. These systems are very similar to several currently being constructed and/or operated, and they serve as baselines for comparison with the less developed systems. Systems 3 through 6 combine the physicochemical systems with the most promising methods of chemically synthesizing food. Glycerol and fructose were individually combined with the physicochemical system to cover the range of nutritional acceptability and process complexity. Ethanol was combined with the glycerol and fructose systems because a moderate amount could probably be consumed by the crew without untoward nutritional or pharmacological effects, and ethanol synthesis is a relatively simple process that could result in weight and power savings.

System 7 utilizes algae for gas exchange; that is, CO₂ removal, CO₂ reduction, and oxygen generation; and as a major food supply. A small amount of food supplements are included to make up for deficiencies in the algae. System 8 was included in the comparison studies to show the effects of adding stored food to an algae system. Should feeding studies show that algae cannot be used for more than 50 percent of the diet, stored food may be required. Excess algae grown to meet the gas exchange requirements of the system is dried and stored in the empty food lockers. System 9 is very similar to 8 except that a physicochemical system provides half of the gas exchange function and glycerol is added in place of stored food. System 10 was included in the study to find out if algae as a gas exchange system alone can compete with the physicochemical systems. Some experimenters have claimed this to be the case.

System 11 assumes that Hydrogenomonas can be used to meet nearly all of the dietary requirements of man. As with the algae system, only a small quantity of food supplements was included in this system. Systems 12 and 13

are Hydrogenomonas versions of systems 9 and 10. System 12 shows the effects of adding glycerol to Hydrogenomonas to obtain possibly a more favorable diet; system 13 inspects the merits of Hydrogenomonas as a CO₂ reduction device alone. System 14, duckweed, is conceptually analogous to systems 7 and 11.

CLOSD ECOLOGICAL SYSTEMS CHARACTERISTICS

To compare the 14 systems, detailed weight, volume, and power data were compiled at the subsystem level and totaled to obtain values for the complete systems. Each system was configured to include all the equipment and expendables required to process man's wastes and provide potable water, food, and oxygen. Certain life support equipment common to all systems were not included, for example, personal hygiene provisions, pressure suits, suit pressure control and ventilation equipment, wash water reclamation, waste collection equipment, radiation protection, personal effects, and emergency equipment.

Prior to assessing subsystem weight, volume, and power, system mass balances were made to establish the methods to be used in handling all materials and to provide data on the quantity of materials each piece of equipment must process. Figures 10 and 11 present the mass balances for systems 3 and

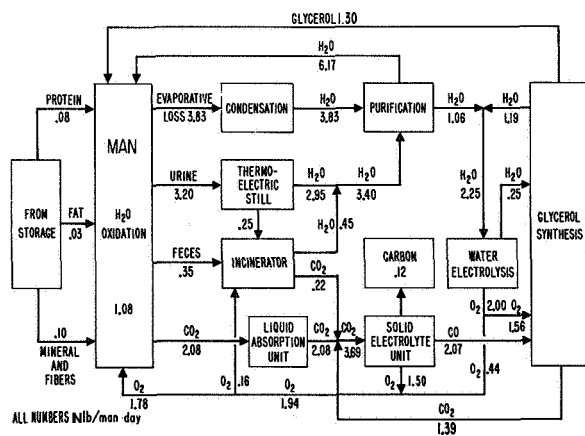


Figure 10.- Mass balance - physicochemical system B with glycerol.

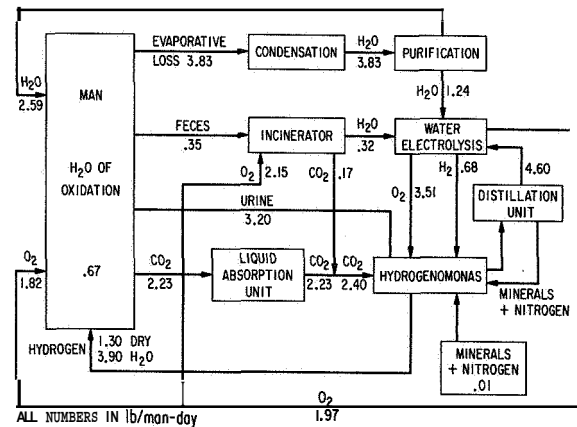


Figure 11.- Mass balance - Hydrogenomonas.

11, respectively. Mass balances for these systems are shown as examples because they ranked first and second in system comparison studies. The first step in preparing the mass balances was the analysis of the diet associated with each system to establish the quantity of each food constituent needed to achieve the required 2800 kcal/man-day, and the oxygen consumed and CO₂ and water produced in metabolizing each constituent. A mass balance for the man was then made to establish water input. For this balance, a urine production of 3.2 lb/man-day and an evaporative loss of 3.83 lb/man-day were assumed for all systems. Fecal production rates varied depending on the diet.

The system process balances were as established by the analysis conducted on each subsystem presented in the preceding sections of this report.

Tables XIII and XIV present the weight, volume, power, and qualitative assessments of reliability, maintainability, safety, cost, and development effort for the Hydrogenomonas and glycerol systems for a crew of 10 men on a one-year mission. Similar system summary data were prepared for all 14 systems for missions of 10-man, one-year and 100-man, three-year.

The CO₂ removal, CO₂ reduction, O₂ generation, contaminant control, water reclamation, and waste processing data are obtained from curves presented in the midterm report and summarized in the physicochemical section of this report. The absolute values listed in the system summary tables may differ from the referenced material because the curves are based on subsystems required to handle all of man's waste products whereas the system mass balances may indicate a particular subsystem need only handle a portion of the load. For example, the water electrolysis cell of system 1 need only produce 75 percent of man's oxygen because the electro dialysis CO₂ removal unit produces enough oxygen to make up the difference and supply the oxygen to the incinerator. In addition, some of the referenced data cannot be directly compared with the system summary tables because the electro dialysis CO₂ removal system has been credited for oxygen production and the Bosch and Sabatier CO₂ reduction systems have been penalized because they require electrolysis to produce oxygen. These credits and penalties were used to allow comparison of subsystem data on a common basis. The tables in the section, however, follow the mass balances and list the subsystem weight, volume, and power that correspond to the actual loading condition.

The weight of stored food was calculated based on the mass balances plus an allowance for packaging and storage. Water supply was included to cover tankage, valves, supply lines, and initial charge. Thermal control estimates were based on the total electrical power consumption of a system plus the metabolic load. Glycerol, fructose, and ethanol data were taken from subsystem studies presented in the section on chemical synthesis of food. Data on algae, Hydrogenomonas, and duckweed are tabulated in the biological systems section.

The last column of each table presents the importance given to a particular subsystem for the qualitative assessments. The number in this column represents the total number of points that can be given in each rating category. For all complete systems, the maximum point count for each category (reliability, maintainability, etc.) was 100.

SYSTEM COMPARISON METHODS

Tables XV and XVI compare the results for 10-man, one-year and 100-man, three-year missions. Before discussing the results of the comparison, a brief description of the comparison methodology will be given. The first two columns present the system number and name. The next three columns present the total system weight, volume, and power data for each system. Columns 6 through 10 present the qualitative assessments of reliability, maintainability, safety, cost, and development effort. Reliability measures the

TABLE XIII.- SYSTEM SUMMARY - PHYSICOCHEMICAL SYSTEM B WITH GLYCEROL

No.	Subsystem	Weight, lb	Vol., %	Power, W	Rel.	Maint	Safety	Cost	Dev.	Max. point count
1	CO ₂ removal	295	5	615	6	6	8	5	4	10
2	CO ₂ reduction	480	36	3,840	7	7	6	7	6	15
3	O ₂ generation	153	2	2,660	6	6	6	7	8	10
4	Thermal control	1,520	87	1,310	8	8	9	8	8	10
5	Contaminant control	233	6	255	8	7	6	7	7	10
6	Water supply	45	2	50	4	5	4	5	4	5
7	Water reclamation	173	9	140	7	6	8	6	6	10
8	Waste processing	300	10	400	8	7	6	6	6	10
9	Glycerol synthesis	485	32	5,480	13	12	14	14	12	20
10	Stored food	950	63	---						
	Total	4,629	277	14,750	67	64	67	65	61	100

TABLE XIV.- SYSTEM SUMMARY - HYDROGENOMONAS

No.	Subsystem	Weight, lb	Vol., %	Power, W	Rel.	Maint.	Safety	Cost	Dev.	Max. point count
1	CO ₂ removal	317	2	600	6	6	8	5	4	10
2	Gas exchange and harvesting	1,300	18	1,400	14	15	13	9	6	20
3	O ₂ generation	240	3	4,120	9	9	9	10	12	15
4	Thermal control	780	41	620	8	8	9	8	8	10
5	Contaminant control	230	6	255	8	7	6	7	7	10
6	Water storage	45	2	50	4	5	4	5	4	5
7	Water reclamation	200	6	250	7	6	8	6	6	10
8	Waste processing	300	10	400	8	7	6	6	5	10
9	Food processing	180	5	1,600	6	7	8	4	2	10
	Total	2,892	96	9,380	70	70	71	60	54	100

TABLE XV. - COMPARISON OF LIFE SUPPORT SYSTEMS FOR MISSIONS EXCEEDING ONE YEAR IN DURATION

MISSION: 10 MEN, ONE YEAR

No	System	Weight, lb	Volume, ft ³	Power, W	Rel. Maint.	Safety	Cost	Dev	Total point count	Total equivalent weight, lb	Weighing factor by			Total rating	Rank
											Points, $\bar{N} = 16.5$	T.E.W., $\bar{N} = 16.5$	Vol., $\bar{N} = 5.5$		
1	Physicochemical system A with stored food	8,020	550	4,500	72	70	74	70	356	8,470	15.6	12.7	5.5	33.8	5
2	Physicochemical system B with stored food	7,920	530	4,500	71	71	70	70	352	8,370	15.8	12.6	5.3	33.7	4
3	Physicochemical system B with glycerol	4,630	230	14,800	67	67	65	61	324	5,760	17.2	8.6	2.3	28.1	3
4	Physicochemical system B with glycerol/ethanol	7,040	400	27,100	65	65	63	59	314	8,840	17.7	13.3	4.0	35.0	6
5	Physicochemical system B with fructose	10,200	600	56,200	64	65	61	56	308	13,300	18.0	20.0	6.0	44.0	12
6	Physicochemical system B with fructose/ethanol	10,100	580	47,800	62	63	59	54	298	12,900	18.5	19.4	5.8	43.7	10
7	Algae--100 percent for gas exchange, 100 percent food	10,900	570	81,200	77	82	66	62	362	15,100	15.3	22.7	5.7	43.7	10
8	Algae--100 percent for gas exchange; 50 percent food, 50 percent stored food	12,200	740	74,000	78	82	67	64	368	16,100	15.0	24.2	7.4	46.6	13
9	Algae--50 percent for food and gas exchange, 50 percent physicochemical system B with glycerol	7,130	340	48,300	70	69	63	58	325	9,900	17.2	14.9	3.4	35.5	7
10	Algae--100 percent for gas exchange, 100 percent stored food	15,000	980	69,200	81	83	69	68	379	18,700	14.4	28.1	9.8	52.3	14
11	Hydrogenomonas--100 percent for CO ₂ reduction, 100 percent food	2,590	96	9,400	70	71	60	54	325	3,390	17.1	5.1	1.0	23.2	1
12	Hydrogenomonas--50 percent for CO ₂ reduction and food, 50 percent physicochemical system B with glycerol	3,440	140	11,800	65	68	59	52	311	4,400	17.8	6.6	1.4	25.8	2
13	Hydrogenomonas--100 percent for CO ₂ reduction, 100 percent stored food	9,880	570	8,300	73	74	62	59	340	10,600	16.4	15.9	5.7	38.0	8
14	Duckweed--100 percent for gas exchange, 100 percent food	9,600	706	77,400	75	82	62	60	354	13,600	15.7	20.4	7.1	43.2	9

TABLE XVI.- COMPARISON OF LIFE SUPPORT SYSTEMS FOR MISSIONS EXCEEDING ONE YEAR IN DURATION

MISSION: 100 MEN, THREE YEARS

No	System	Weight, lb	Volume, ft ³	Power, W	Rel	Safety	Cost	Dev.	Total point count	Total equiv- alent weight, lb	Weighing factor by			Total rating	Rank
											Points $\bar{N} = 16.5$	T.E.W. $\bar{N} = 16.5$	Vol. $\bar{N} = 5.5$		
1	Physicochemical system A with stored food	208,700	15,000	41,100	72	70	74	70	356	211,000	15.6	11.1	11.1	23.0	11
2	Physicochemical system B with stored food	208,300	15,000	39,800	71	70	70	70	352	211,000	15.8	27.1	11.1	54.0	12
3	Physicochemical system B with glycerol	58,500	3,300	114,400	67	64	65	61	324	63,800	17.2	7.6	2.5	27.3	3
4	Physicochemical system B with glycerol/ethanol	92,600	4,980	252,900	65	62	63	59	314	103,000	17.7	12.0	3.7	33.4	7
5	Physicochemical system B with fructose	114,645	6,330	529,600	64	62	61	56	308	133,000	18.0	14.9	4.7	37.6	8
6	Physicochemical system B with fructose/ethanol	119,400	6,450	453,300	62	60	59	54	298	135,000	18.5	15.5	4.8	38.8	9
7	Algae--100 percent for gas exchange, 100 percent food	85,900	4,500	790,400	77	75	66	62	362	110,000	15.3	11.1	3.3	29.7	5
8	Algae--100 percent for gas exchange; 50 percent food, 50 percent stored food	168,800	10,900	720,700	78	77	67	64	368	192,000	15.0	21.9	8.1	45.0	10
9	Algae--50 percent for food and gas exchange; 50 percent physicochemical system B with glycerol	65,100	3,200	463,500	70	65	63	58	325	82,000	17.2	8.5	2.4	28.1	4
10	Algae--100 percent for gas exchange, 100 percent stored food	264,200	18,000	673,000	81	78	69	68	379	286,000	14.4	34.3	13.4	62.1	14
11	Hydrogenomonas--100 percent for CO ₂ reduction, 100 percent food	37,700	660	103,300	70	70	60	54	325	38,600	17.1	4.9	0.5	22.5	1
12	Hydrogenomonas--50 percent for CO ₂ reduction and food, 50 percent physicochemical system B with glycerol	34,400	1,040	119,700	65	67	59	52	311	39,900	17.8	4.5	0.8	23.1	2
13	Hydrogenomonas--100 percent for CO ₂ reduction, 100 percent stored food	231,400	15,000	93,800	73	72	62	59	340	236,000	16.4	30.1	11.1	57.6	13
14	Duckweed--100 percent for gas exchange, 100 percent food	79,300	1,500	751,900	75	75	62	60	354	103,000	15.7	10.3	4.2	30.2	6

relative ability of a system to provide the required function throughout the mission. Maintainability measures the amount and type of maintenance required to keep this system in operation. The safety rating indicates the seriousness of a failure—fire, explosion, cabin contamination, etc. Cost reflects the needs of research, development, engineering, fabrication, and qualification of the system. The development rating measures the magnitude of the development effort other than cost, that is, probability of success, schedule, availability of special talents, resources, and facilities. The total point count, in the next column, is the sum of the five rated categories. Total equivalent weight combines weight and power by applying a power penalty in accordance with a curve fitted to three points, namely, 10 kW require 100 lb/kW, 100 kW require 50 lb/kW, and 1000 kW require 30 lb/kW. The power penalty varies with the total electrical load of the system. The three columns following the total equivalent weight column form the basis for final rating. The total point count, total equivalent weight, and volume are weighed on a 3:3:1 basis, respectively. Volume is considered one-third as important because it is generally a direct function of weight. It is included in the ratings, however, to measure the effects of any extremely high or low density systems. The numbers in these three columns were established by dividing the volume numbers for the 10-man, one-year mission by 100, as an arbitrary starting point. The average of these numbers was 5.5, hence, the $N = 5.5$ at the top of the volume rating column. To provide three times the importance to the point count and total equivalent weight, an average of 16.5 was established for them, hence, $N = 16.5$ at the top of those columns. This then means that the average of the numbers in that column must be 16.5. It is then determined what number the average total equivalent weight and point count must be divided by to give 16.5. That number then provides a basis for converting all the total equivalent weights and point counts into numbers that can be compared to each other and to the volume numbers all in the ratio of 3:3:1. The only remaining problem is to invert the point count rating because high point count is an advantage while high total equivalent weight and volume are disadvantages. This is accomplished by replacing the normalized point count numbers by the number (33-point count). This methodology allows direct comparison of the three variables. The total system rating is obtained by adding the point count, total equivalent weight, and volume ratings. The rank column lists the 14 systems in the preferred order; low total rating numbers are best.

SYSTEM SELECTION

The system comparison results are summarized in the following list. The systems are listed in order of preference as established by the system ratings presented by tables XV and XVI.

10-Man, one-year mission

1. Hydrogenomonas
2. Physicochemical system with glycerol
3. Physicochemical system with stored food
4. Physicochemical system with glycerol/ethanol
5. Photosynthetic systems (algae, duckweed)

100-Man, three-year mission

1. Hydrogenomonas
2. Physicochemical system with glycerol
3. Photosynthetic systems (algae, duckweed)
4. Physicochemical system with glycerol/ethanol
5. Physicochemical system with stored food

Hydrogenomonas and glycerol stand out as concepts that offer greater potential as food-producing systems for the missions studied. Many problems outlined previously in this report and discussed further in the research and development problem section are yet to be solved. Interestingly, the physicochemical system with stored food is relatively attractive for the shorter mission of one year. Ethanol synthesis did not measure up to expectations for either mission, but may still offer some advantages as a companion to other systems. A long mission is required to bring the photosynthetic systems into third place. It appears that unless serious problems are encountered with the Hydrogenomonas and glycerol systems, algae and duckweed studies should not be accelerated.

RESEARCH AND DEVELOPMENT PROBLEMS

On the basis of the comparison studies and the problem areas identified during concept analysis, twelve research and development areas have been identified. These twelve areas are considered those most desirable to develop in order to evolve an extended life support system that incorporates the manufacture of food from wastes. These twelve areas embrace more than one type of system and have different priorities. The choice of systems to study is based mainly on probability of success; however, more than one system is recommended for investigation for three reasons: (1) the great uncertainty associated with many aspects of these systems make it very likely that future work will reverse orders of preference shown in the previous section, (2) the long lead time required for these systems demands that initial work begin now if systems are to be flight-ready in the 1980's, and (3) in some areas, moderate expenditures can be expected to settle important questions.

BASIC PROGRAM PLAN

The basic program plan, presented in figure 12, outlines a four-year research and development effort. Hydrogenomonas work has been emphasized because of its first order rating in the system studies. The Hydrogenomonas culture and nutrition studies have been scheduled to be conducted simultaneously because of their interdependency and are to be completed in three years. The Hydrogenomonas prototype development overlaps by six months the

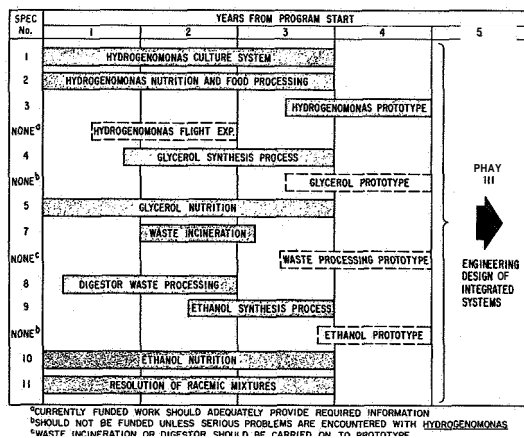


Figure 12. Basic program schedule.

culture and nutrition work in order to reduce overall program schedule. By this time in the development effort, Hydrogenomonas system requirements should be well established and the probability of encountering serious problems during the last six months of the culture and nutrition studies is relatively small. If all goes reasonably well, phase III can begin four years after phase II go-ahead. The next scheduled item is a Hydrogenomonas flight experiment planned to study Hydrogenomonas under weightlessness to look for growth anomalies. It is understood that current NASA plans include such a flight experiment; therefore, it was not included in the cost estimate presented by year at the bottom of figure 12.

Glycerol synthesis work was included in the program because of its favorable rating in the system studies and the desire to have a backup program in case Hydrogenomonas studies show negative results. It is also felt to be distinctly possible that a mixed diet of the two will be superior to either separately. Glycerol process and glycerol nutrition studies are included in the program. The glycerol process work is scheduled to begin 10 months after the glycerol nutrition work to allow time for some tentative conclusions concerning glycerol as a food source before process work has been carried very far. Such a schedule should allow the glycerol nutrition and process work to be completed at approximately the same time. The glycerol prototype development has not been described by a specification because it probably should not be funded unless serious problems are encountered with Hydrogenomonas.

Research and development efforts on the incineration and aerobic digester waste-processing systems are planned to establish the best method of integrating them with a biological life support system, particularly Hydrogenomonas, and then to select the better approach for further development. The aerobic digester work precedes the incineration work because of the longer time required to complete the digester work. It is desirable to delay the start of the waste processing system work as long as possible to provide a good definition of the wastes to be processed, but yet finish this work soon enough to leave ample time for Hydrogenomonas culture studies that use the output data from the waste studies. The selected waste-processing system should be carried on to a flight prototype development. This prototype development has not been covered by a specification since the process has not been selected.

Ethanol development has been included in the basic program plan because there is a strong likelihood that glycerol cannot be used as a source of more than 50 percent of the caloric requirements of man, and ethanol presents a relatively expedient means of supplementing glycerol. The ethanol nutrition studies are scheduled to begin early because of their duration and the need to answer basic questions as to the compatibility of glycerol and ethanol. The ethanol process work is scheduled late in the program to allow time to establish the value of ethanol as a companion to glycerol or fructose prior

to conducting any process studies. The ethanol prototype development, like the glycerol prototype development, has not been covered by a specification. It should not be funded unless serious problems are encountered with Hydrogenomonas.

The resolution of racemic mixtures has been included in the program because **it** is the key to the manufacture of higher sugars and other carbohydrates.

HYDROGENOMONAS

Generally, this organism has been studied only in very **small** amounts, for example, 10 ml. Substantial inconsistencies in gas exchange stoichiometry are reported. Growth requirements are not well specified. Nutrition studies are just now beginning. The ability of Hydrogenomonas to accept human wastes is not well delineated. Space cabin processing problems are hardly defined, certainly not solved. A first necessity is to culture enough of the organism to begin large scale studies. As soon as sufficient quantities of the organism are available, two simultaneous research programs should begin, one in feeding and the other in growth as a function of environment. ~~When~~ and if these two programs appear reasonably successful, probably not before two years, the performance of specific space systems, long term reliability and response to specific space stresses will require study. The research and development problems listed below are those necessary to carry a Hydrogenomonas system to flight readiness. Such a list must be very tentative with respect to all but initial studies.

- A. Development of continuous culture apparatus capable of producing Hydrogenomonas in large quantities
- B. The study of growth versus environment
 - 1. Study growth rate, composition, and gas production as a function of:
 - Total pressure and influx gas composition
 - Temperature and pH
 - Amount and source of nitrogen, particularly using urine
 - Amount and source of minerals and trace substances
 - 2. Determine effects of recycling nutrient medium
 - 3. Determine types of contaminants and their effects
- C. Nutrition work
 - 1. Animal studies followed by human studies of:
 - Dietary insufficiencies and required supplements
 - Toxicity, especially high nucleic acid content and poly-p-hydroxybutyric acid

Digestibility
Excretion products

2. Processing required before consumption

D. Flight system development. The main problems will include:

Gas feed accuracy
Urine processing
The feed of incinerator products
The effects of weightlessness and radiation
Required nutrient recycling and processing

GLYCEROL

So far, the literature has not revealed records of prolonged human feeding at more than 110 g/day. At this level, it was apparently acceptable. Animal work revealed problems when glycerol constituted 40 to 60 percent of the diet. Osmotic problems seem basic. Consequently, nutrition work is required. Because it will probably be several years before glycerol nutritional work has produced a definitive answer, simultaneous study of processing problems is advocated. Generally, there are two attractive processes for formaldehyde synthesis from CO and H₂ and two attractive processes for glycerol synthesis from formaldehyde. As might be expected, the two less-studied alternatives are theoretically capable of better results.

Which processes should be studied or whether all four should be studied is a question of schedule and funds available. The problem areas given below are basically applicable to all four processes.

A. Nutrition work, animal and human, covering:

Maximum utilized satisfactorily
Toxicity
Digestibility and absorption phenomena
Preparation with stored food supplements

B. Processing studies including:

Determination of equilibria and kinetics
Determination of by-products as a function of reaction conditions
Separation of desired reactants
Design of energy-handling techniques
Selection and study of catalysts
For electrochemical steps, determination of optimum voltage, electrode, and current density
Definition of effects of zero g upon energy and mass transfer requirements
Development of lightweight, reliable equipment

ALGAE

As a gas exchange system, algae has been studied at considerable length for space applications as well as for other reasons. Precise information about growth as a function of environment, however, is still lacking. Algae has been demonstrated to be very nearly a complete nutrient for humans, but digestibility problems and unpleasant taste remain formidable obstacles. Presently, great drawbacks to the use of algae are the very considerable power and associated equipment requirements when artificial light is used and the heavy apparatus necessary to provide natural light; many space missions involve substantial periods of time out of sight of the sun. Even if a 100-percent algae diet proves feasible, it is very doubtful that an algae system will prove more attractive than a physicochemical system carrying stored food for missions of duration less than two years unless present estimates (themselves optimistic) of 7 kW of electric power per man can be reduced. The standout problems with algae are processing to increase digestibility and increasing the efficiency of power distribution and utilization. Even though grave doubts exist as to the applicability of algae to space missions, the availability of more or less flight-ready systems suggest early tests of such systems in space to determine the nature of zero g processing problems.

All of the problems listed above under B, C, and D for Hydrogenomonas require study with respect to algae also. For algae systems, however, processing to increase digestibility is, by far, the most immediate requirement. In addition, research and development requirements associated with algae lighting are:

- Algae storage in the dormant state

- Methods of increasing the efficiency of electric to light energy transition

- Methods of increasing the efficiency of light distribution including the use of fiber optics

- The use of collectors and/or mirrors to bring natural light to algae

- The use of radioisotope-phosphor systems to provide algae illumination

DUCKWEED

Duckweed has very nearly all of the same research and development requirements as algae, including the important necessities for increasing digestibility and increasing the efficiency of power distribution and utilization. Duckweed has not been studied as long or as extensively as algae. Duckweed has a more uncertain prognosis than algae under weightlessness because of more gravity-influenced growth factors. Nutrition studies with duckweed are very sparse; consequently, nutrition studies of a more basic nature would be required at first, relative to algae. Unless duckweed is shown to be substantially better than algae from a nutritional viewpoint, further work on duckweed does not seem justified.

LITTLE STUDIED MICROBIOLOGICAL SYSTEMS

Although studies of this topic did not reveal any new system that seemed worth recommending for a specific research and development plan, enough possibilities were discovered to result in the recommendation that a two- to three-man-year effort be funded to survey the entire microbiological area more thoroughly to investigate such topics as anaerobic and aerobic digestion of waste, other autotrophic organisms that could be consumed as food, and the use of yeasts or bacteria to consume glycerol manufactured physicochemically to produce food of a more acceptable and nutritionally complete nature than glycerol.

THE INTEGRATION OF WASTE INCINERATION OR AEROBIC DIGESTION WITH BIOLOGICAL SUBSYSTEMS

Incineration

The incineration of a variety of spacecraft wastes, including human fecal matter, has been studied both analytically and experimentally for a number of years. Prototype models have been built and tested and many of the flight system problems resolved. The work to date has been directed toward the waste-burning problem and methods of reducing the weight, power, and oxygen loss associated with this process. The use of physicochemical regenerative life support systems with stored food provides sufficient stored materials to allow venting of the incineration products without serious weight penalties. The biggest single advantage of biological systems is that of potentially closing the food loop. This potential can only be effectively realized by recovering the products of incineration and using them as nutrients for the biological organism.

The primary products of incineration, that is, CO_2 and water vapor, can be readily utilized by any of the biological organisms currently being considered for spacecraft application. The breakdown and oxidation of organic compounds, such as urea and amino acids (especially sulphur-containing amino acids) and inorganic compounds such as phosphates and oxalates, however, will produce substances *that* may be toxic to the biological system or man. The incineration process particularly should be studied in greater detail to determine the chemical products and the effects of these products on Hydrogenomonas.

Aerobic Digestion

A considerable amount of work has been done to define the characteristics of aerobic digestors. These studies have, in general, defined a range of preferred digestion periods, waste loading factors, and reduction of total sludge solids and chemical oxygen demand to be expected in operating an

aerobic digester. The most important conclusion reached in the previous studies is that aerobic digestion does not greatly reduce the total insoluble solids present in the slurry, but stabilizes the wastes by converting biologically active organic matter into inorganics and organic materials resistant to decomposition. Approximately 15 to 20 percent reduction in raw waste solids can be expected. It is then obvious that without further processing the aerobic digester is of little value to the closed ecological system since much of the remaining solid matter is unusable by Hydrogenomonas or algae. The aerobic digester may, however, be an important first step in the waste recovery system to be followed by incineration, further biological reduction, or processing as a food supplement for man. A program, based on this previous aerobic digester work, that explores these latter steps in waste processing as well as the compatibility of the effluent from these processes with Hydrogenomonas is required.

ETHANOL

Nutrition

The overall objective of the nutrition research program is to determine the maximum amount of ethanol that can be ingested by man for a one- to three-year period, and to determine the variation of side effects as a function of frequency and manner of administration, size of dose, duration of ethanol feeding, and the nature of the remainder of the diet.

Since acute human thresholds for pharmacologic effects are presently rather well defined, the immediate objective is to determine the effects, if any, of extended feeding of subthreshold amounts. This would be done first with two or more types of mammals and later with human subjects. Simultaneously, tracer experiments with animals would further elucidate the metabolic pathways of ethanol. It would also be desirable to study the compatibility of ethanol with glycerol, fructose, and other substances likely to be present in a synthetic space diet.

Process Studies

Previous research and development efforts in ethanol production are largely proprietary, as a large and expanding market exists for this much used organic solvent. At least seven methods for industrial ethanol production, including the Fischer-Tropsch reaction, have been reported. For commercial scale production, low cost is the dominant criterion in process selection, so the direct application of these methods to an extended mission life support system is doubtful. In the present commercial process, about 80 percent conversion to alcohols is obtained, but ethanol is only 12 percent of the alcohols. In addition to increasing the low yield of this process, the separation of ethanol from the by-products presents an area for development work. Present weight estimates for a spacecraft ethanol synthesis system are

greater than an equivalent glycerol system, but experimentation might easily yield results that reverse these estimates.

THE RESOLUTION OF RACEMIC MIXTURES

As mentioned elsewhere in this report, there is a good chance that a simple nonoptically active three-carbon compound, namely, glycerol, will prove a satisfactory major calorie source. Should it prove necessary to provide a tetrose or any more complicated carbohydrate, then it will probably be necessary to provide the D form, either by asymmetric synthesis or by performing a resolution of a racemic mixture into the D and L forms since normal nonbiological methods of preparation of carbohydrates result, not in an optically active form, but in the racemic mixture. The synthesis of D glyceraldehyde is not only typical of this type of problem but would also lead directly to the straightforward synthesis of the D form of higher carbohydrates from D glyceraldehyde.

Of the five known methods for the resolution of racemic mixtures, the approach tentatively proposed for a research program is the chromatographic resolution. The yields are expected to be high and the possibility of automating the device is good.

It is also recommended that an attempt be made at direct synthesis of the D form of glyceraldehyde using an optically active catalyst.

SUBSEQUENT PHASES

The program plan calls for the engineering, design, manufacture, assembly, checkout, and testing of the closed ecological system that emerges from the research and development phase as the most promising for use in manned space missions of one-year or greater duration. The research and development phase has been planned to provide flight prototype subsystems for those system functions which manufacture food and process man's wastes. In the case of Hydrogenomonas, it has been assumed that parallel research and development will provide those physicochemical subsystems required to accomplish the regeneration of perspired and expired water vapor and the collection and concentration of CO₂. Specifically, a water electrolysis cell, CO₂ removal unit, and a water reclamation device are required. It is reasonable to assume current work on these subsystems will provide flight prototype units by the conclusion of the research and development phase of this program. Additional subsystems will also be required to complete the system, for example, thermal control, humidity control, contaminant removal, urine and feces collection, water storage and delivery, and personalhygiene. These subsystems are being developed as part of a physicochemical life support system currently being readied for testing at NASA-Langley. The concepts used in the Langley system are, for the most part, applicable to longer missions and therefore much of the development work will be of direct value in the design and fabrication

of the longer duration system. However, a great deal of integration of these subsystems with the food manufacturing subsystems will be necessary.

ENGINEERING (PHASE 111)

The engineering design phase will be based on subsystem prototype development work. The primary problem will be to determine the methods of integrating these subsystems, to identify the equipment required to achieve this integration, and to establish modifications of the subsystems required to provide compatibility of design. Specific tasks required to complete the engineering phase are listed below.

Review subsystem development work to document subsystem characteristics, specific design details, and any deficiencies in performance.

Conduct detailed system integration analysis to define the methods of integrating the subsystems, to match the subsystem requirements with the prototype subsystem capabilities, and to determine specific redesign and new design areas.

Accomplish the subsystem redesign and design the new system elements identified in the previous task.

Conduct system layout to determine packaging methods; subsystem groupings; bracketry, electrical harness and instrumentation design, and plumbing routing.

Prepare detailed drawings of all system elements and assembly drawings.

A detailed schedule and cost estimate by task has not been prepared at this time because it is, of course, very dependent upon the results of the research and development phase. It is anticipated that nine months will be required to accomplish the task outlined above.

MANUFACTURING (PHASE IV)

The manufacturing phase of the program will be based on the engineering drawings and specifications prepared in the design phase. Some equipment developed in the research and development phase can be used directly or modified for use in the prototype ecological system. Many of the subsystems will not be available from this or other programs and must, therefore, be fabricated during this phase. It is anticipated that considerable amounts of the system fabrication will be subcontracted to organizations familiar with the design, fabrication, and testing of particular components or subsystems. This subcontract effort will increase the problems of integrating system requirements, design interfaces, fabrication and assembly techniques, and system checkout.

The manufacturing phase will include subsystem functional checkout, assembly of the integrated system and a combined systems checkout to demonstrate compliance with system requirements. It is estimated that the manufacturing phase of the program will require 14 months.

PROTOTYPE SYSTEM EVALUATION (PHASE V)

The prototype ecological system in an environmental chamber with other prototype spacecraft systems is particularly difficult to evaluate. Depending on the length of such testing, the number of subjects used, the amount and type of monitoring required, and data reduction system employed, costs can vary widely. Langelly life support systems personnel are currently studying the problem of manned system evaluation in an attempt to optimize information obtained and costs required to obtain it. Long-duration manned testing is very expensive; the cost will depend on the sophistication of the systems and data-handling techniques. It is anticipated that phase V of this program will involve several short-duration tests ranging between 2 and 30 days to accomplish shakedown of the equipment, calibration of instrumentation, and refinement of operating procedures. Long-duration tests will then follow.

Detailed planning of phase V should await completion of the research and development and engineering phases when the details of the system to be tested are known and the objectives of the evaluation phase can be better defined.

CONCLUSIONS

The review of state-of-the-art and proposed physicochemical and biological systems, and the subsequent analyses of these approaches to life support, led to a number of important conclusions.

Of all of the physicochemical subsystems other than food synthesis subsystems examined, the greatest advantages were offered by the electro-dialysis and liquid absorption CO_2 removal subsystems, the Bosch and solid electrolyte cell CO_2 reduction subsystems, the porous electrode-absorbent matrix and ion exchange membrane water electrolysis cells, and the waste heat and thermoelectric vacuum waste water distillation units. These physicochemical subsystems are generally much lighter and consume less power than their biological equivalents. The primary drawback of the currently developed physicochemical systems is that they do not manufacture food.

The study of the chemical synthesis of food identifies glycerol, ethanol, and fructose as promising food sources considering the balance between nutritional requirements and process feasibility. Glycerol nutrition studies are not definitive, but it is anticipated that glycerol can satisfy as much as half of man's daily caloric requirements. The approach to glycerol synthesis evolved during the study involves the step-wise formation of methanol,

formaldehyde, an ethanol solution of trioses, and glycerol. Ethanol was considered as an expedient means of supplementing the glycerol diet. The pharmacological effects of ethanol can be avoided by controlling the rate of intake, and the relatively straightforward processes involved in the manufacture of ethanol from CO and H₂ make it an attractive candidate. The nutritional acceptability of fructose is better established than that of glycerol or ethanol; and fructose is, perhaps, more compatible with ethanol than is glycerol, but the synthesis of fructose involves the same steps as glycerol plus several more, including a difficult resolution of a racemic mixture.

The review of biological life-support concepts included the more studied algae, Hydrogenomonas, and duckweed concepts as well as concepts employing a number of little studied or unstudied biological organisms. Information on all but the three established concepts was so meager that detailed comparisons of new concepts were impossible.

Fourteen closed ecological systems comprised of various physicochemical, including chemical food synthesis, and biological subsystems were compared to select preferred systems. The results of the comparison studies are listed below. Systems are listed in the order of preference.

<u>10-Man, One-Year Mission</u>	<u>100-Man, Three-Year Mission</u>
1. <u>Hydrogenomonas</u>	1. <u>Hydrogenomonas</u>
2. Physicochemical system with glycerol	2. Physicochemical system with glycerol
3. Physicochemical system with stored food	3. Photosynthetic systems (algae, duckweed)
4. Physicochemical system with glycerol/ethanol	4. Physicochemical system with glycerol/ethanol
5. Photosynthetic systems (algae, duckweed)	5. Physicochemical system with stored food

Hydrogenomonas and glycerol offer sizable advantages for the missions considered. A considerable amount of work must be done to answer many questions concerning these systems. To that end, nutrition and growth studies of Hydrogenomonas and nutrition and process studies of glycerol are recommended. The physicochemical system with stored food appears relatively competitive for the one-year mission, but is as much as five times the weight of other systems for three-year missions. The use of ethanol to supplement glycerol did not prove to be as attractive an approach as anticipated. However, because ethanol process work has such a high probability of success, limited investigations in this area are recommended. The photosynthetic systems suffer the disadvantages of large weight penalties associated with lighting the culture panels. Large culture volumes, light bulbs, ballast, and controls add substantially to the system weight and power. Fructose process weight, power, and complexity resulted in an unfavorable position in the system comparison studies for this concept.

A research and development plan calling for major efforts in Hydrogenomonas, glycerol, and waste processing and minor efforts in algae nutrition and lighting, ethanol, and the resolution of racemic mixtures was prepared. A basic four-year research and development program was outlined.

BIBLIOGRAPHY

Basic Nutritional Requirements

Conference on Nutrition in Space and Related Waste Problems. NASA SP-70, 1964.

Chittenden, R.: *Physiological Economy in Nutrition with Special Reference to the Minimal Protein Requirements of the Healthy Man: An Experimental Study*. F. A. Stokes Co., New York, 1904.

Hegsted, D. M.: *Proteins in Space Nutrition*. NASA SP-70, 1964, pp. 135-141.

Sherman, H. C.: *Chemistry of Food and Nutrition*. Eighth ed., The Macmillan Co., New York, 1952, p. 29.

Glycerol Feeding Studies

Deichmann, W. : *Glycerol; Behavior in Animal Organism (Review of Literature)*. Ind. Med., Ind. Hyg. Sect., vol. 1, Oct. 1940, pp. 60-67.

El Mofty, A.; Khattab, M.; and Abou Issa, H. M.: *Glycerol Metabolism in Man in Health and Diabetes*. J. Chem. U. A. R., vol. 4, 1961, pp. 41-53 (C.A. 56: 10800h).

Gil'miyarova, F. N.: *Metabolism of Free Glycerol in the Animal Organism*. Tr. Kuibyshevsk. Med. Inst., vol. 29, 1964, pp. 10-18 (C.A. 62: 874g).

Johnson, V.; Carlson, A. J.; and Johnson, A.: *Studies on Physiological Action of Glycerol on Animal Organism*. Am. J. Physiol., vol. 103, March 1933, pp. 517-534.

Lundquist, Frank; Tygstrup, Niels; Winkler, Kjeld; and Jensen, K. Birger: *Glycerol Metabolism in the Human Liver: Inhibition by Ethanol*. Science, vol. 150, no. 3696, Oct. 29, 1965, pp. 616-617.

Rose, Irwin A.; Kellermeyer, R.; Stjermholm, R.; and Wood, H. G.: *Distribution of C¹⁴ in Glycerol from Deuterated Glycerol-C¹⁴ as a Measure of the Effectiveness of Triosephosphate Isomerase in Vivo*. J. Biol. Chem., vol. 237, 1962, pp. 3325-3331.

Ethanol Feeding Studies

Anton, A. H.: *Ethanol and Urinary Catecholamines in Man*. Clin. Pharmacol. Therap., vol. 6, July-Aug. 1965, pp. 462-469.

Freund, Gerhard; and O'Holloren, Paul: *Acetaldehyde Concentrations in Alveolar Air Following a Standard Dose of Ethanol in Man*. J. Lipid Res., vol. 6, no. 4, 1965, pp. 471-477.

- Isselbacher, K. J.; and Greenberger, N. J.: Metabolic Effects of Alcohol on the Liver. *New Engl. J. Med.*, vol. 270, Feb. 1964, pp. 351-356, 402.
- Keller, A. Z.; and Terris, M.: The Association of Alcohol and Tobacco with Cancer of the Mouth and Pharynx. *Am. J. Public Health*, vol. 55, Oct. 1965, pp. 1578-1585.
- Lanigan, M. Regina; and Anderson, K. E.: Action of Antimetabolites on the Growth of *Desulfovibrio Desulfuricans*. *Sci. Studies, St. Bonaventure Univ.*, vol. 21, 1963, pp. 80-98 (C.A. 60: 16248a).
- Magee, H. E., et al.: (Symposium) *Proc. Nutr. Soc. (Great Britain)*, vol. 14, 1955, p. 93.
- Tygstrup, N.; Winkler, K.; and Lundquist, F.: The Mechanism of the Fructose Effect on the Ethanol Metabolism of the Human Liver. *J. Clin. Invest.*, vol. 44, May 1965, pp. 817-830.
- Westerfield, W. W.; and Schuleman, M. P.: Metabolism and Caloric Value of Alcohol. *J. Am. Med. Assoc.*, vol. 170, no. 2, May 1959, pp. 197-203.

Fructose Feeding Studies

- Holdsworth, C. D.; and Dawson, A. M.: Absorption of Fructose in Man. *Proc. Soc. Exptl. Biol. Med.*, vol. 118, Jan. 1965, pp. 142-145.
- Miller, M.; Craig, J. W.; Drucker, W. R.; and Woodward, H., Jr.: The Metabolism of Fructose in Man. *Yale J. Biol. Med.*, vol. 29, Dec. 1956, pp. 335-360.
- Strub, I. H.; Best, W. R.; Consolazio, C. F.; and Grossman, M. I.: Utilization of Intravenously Injected Fructose and Invert Sugar in Normal Human Subjects. *J. Clin. Nutrition*, vol. 2, no. 1, Jan.-Feb. 1954, pp. 32-37.
- Tygstrup, N.; Winkler, K.; and Lundquist, F.: The Mechanism of the Fructose Effect on the Ethanol Metabolism of the Human Liver. *J. Clin. Invest.*, vol. 44, May 1965, pp. 817-830.
- Van Itallie, T. B.; and Shull, K. H.: Effect of Fructose Feeding on Glucose Tolerance in Man. *J. Lab. Clin. Med.*, vol. 50, no. 3, Sept. 1957, pp. 391-399.
- Winitz, M.; Graff, J.; and Seedman, D. A.: Effect of Dietary Carbohydrate on Serum Cholesterol Levels. *Arch. Biochem. Biophys.*, vol. 108, Dec. 1964, pp. 576-579.

Miscellaneous Nutritional Topics

- Recommended Dietary Allowances. NAS-NRC Pub. 1146, Sixth rev. ed., 1964.
- Cathcart, E. P.; and Markowitz, J.: The Influence of Various Sugars on the Respiratory Quotient. A Contribution to the Significance of the Respiratory Quotient. *J. Physiol.*, vol. 63, 1927, pp. 309-324.
- Cori, Carl F.; and Cori, Gerty T.: The Fate of Sugar in the Animal Body. VIII. The Influence of Insulin on the Utilization of Glucose, Fructose, and Dihydroxyacetone. *J. Biol. Chem.*, vol. 76, 1928, pp. 755-795.
- Drews, J.; and Fölsch, E.: Hemmung des Glycineinbaus in Tumorzellprotein durch Glycerinaldehyd. *Naturwiss.*, vol. 51, 1964, pp. 440-441.
- Grieshaber, H.: Beitrag zum Verhalten der Sorbose im Stoffioechsel; die Resorption und Ausscheidung der Sorbose und ihre Wirkung auf Blut und Harnzucker. *Z. Klin. Med.*, vol. 129, 1936, pp. 412-422.
- Hall, W. K.; Doty, J. R.; and Eaton, A. G.: The Availability of dl-threonine and dl-allothreonine for the Formation of Carbohydrate. *Am. J. Physiol.*, vol. 131, 1940, pp. 252-255.
- Lanigan, M. Regina; and Anderson, K. E.: Action of Antimetabolites on the Growth of *Desulfovibrio Desulfuricans*. *Sci. Studies, St. Bonaventure Univ.*, vol. 21, 1963, pp. 80-98 (C.A. 60: 16248a).
- Lardy, Henry A.; Wiebelhaus, Virgil D.; and Mann, Kingsley M.: Mechanism by which Glyceraldehyde Inhibits Glycolysis. *J. Biol. Chem.*, vol. 187, 1950, pp. 325-337.
- McCollum, E. V.; Simmonds, Nina; Shipley, P. G.; and Park, E. A.: Studies on Experimental Rickets. XII. Is There a Substance other than Fat-soluble A Associated with Certain Fats which Plays an Important Role in Bone Development? *J. Biol. Chem.*, vol. 50, 1922, pp. 5-30.

Formaldehyde Synthesis

- Akerlof, G.; and Mitchell, P.: Regeneration of Carbohydrates in a Closed Circuit Respiratory System. Presented at AIAA Meeting, Los Angeles, May 2, 1963.
- Ewell, R. H.: Calculation of Chemical Equilibrium at High Pressures. *Ind. Eng. Chem.*, vol. 32, 1940, pp. 147-153.
- Faith, W.; Keyes, D.; and Clark, R.: *Industrial Chemicals*. Second ed., John Wiley and Sons, Inc., New York, 1957.
- Jones, E., and Fowlie, G.: Thermodynamics of Formaldehyde Manufacture from Methanol. *J. Appl. Chem. (London)*, vol. 3, 1953, pp. 206-313.

- Kirk, R.; and Othmer, D., eds.: Encyclopedia of Chemical Technology, Vol. 9. Interscience, New York, 1952, pp. 31-60.
- Newton, R. H.; and Dodge, Barnett F.: Activity Coefficients of Gases. Application to Calculation of Effect of Pressure on Homogeneous Chemical Equilibria and to Calculation of Integral Joule-Thomson Effects. Ind. Eng. Chem., vol. 27, 1935, pp. 577-581.
- Newton, R. H.; and Dodge, Barnett F.: The Equilibrium Between Carbon Monoxide, Hydrogen, Formaldehyde and Methanol. II. The Reaction $\text{CO} + 2\text{H}_2 \rightleftharpoons \text{CH}_3\text{OH}$. J. Am. Chem. Soc., vol. 56, 1934, pp. 1287-1291.
- Robell, A. J.: Personal consultation with P. Van Rysselberghe, Aug. 12, 1965.
- Scholten, J., et al.: Kinetics and Mechanism of the Decomposition of Formic Acid and of the Watergas Reaction over Alumina and Magnesia. Proc. 3rd Intl. Congress on Catalysis, North-Holland Pub. Co. (Interscience), Amsterdam, vol. 2, 1965, pp. 882-900.
- Shearer, R.; King, J.; and Palladino, C.: Electrolytic Production of Oxygen and Reduction of CO_2 . First Quarterly Rep., Contract No. NASr-70, MSA Research Corp., Callery, Pa., Jan. 12, 1962.
- Shearer, R.; King, J.; and Palladino, C.: Electrolytic Production of Oxygen and Reduction of CO_2 . Second Quarterly Rep., Contract No. NASr-70, MSA Research Corp., Callery, Pa., Apr. 24, 1962.
- Sinyan, Uy. Ye.: The Possibility of Physicochemical Synthesis of Carbohydrates in a Spaceship Cabin. Probl. Space Biology, vol. 3, June 29, 1964, pp. 401-409.
- Teeter, T. E.; and Van Rysselberghe, Pierre: Reduction of Carbon Dioxide on Mercury Cathodes. J. Chem. Phys., vol. 22, 1954, pp. 759-760.
- Teeter, T.; and Van Rysselberghe, P.: Reduction of Carbon Dioxide on Mercury Cathodes. Proc. 6th Meeting, Intl. Comm. Electrochemistry, Thermo., and Kinetics (CITCE), Butterworths, London, 1955, pp. 538-542.
- Walker, J.: Formaldehyde. Third ed., Reinhold, New York, 1964.

The Chemical Synthesis of Proteinoids

- Fox, S. W.: Organic Geochemistry. Pergamon Press, New York, 1963.
- Harada, Kaoru; and Fox, Sidney W.: Thermal Synthesis of Natural Amino-Acids from a Postulated Primitive Terrestrial Atmosphere. Nature, vol. 201, no. 4917, Jan. 24, 1964, pp. 335-336.
- Miller, S. A.: Nutrition in Space and Related Waste Problems. University of South Florida, Tampa, Florida, 1964, p. 343.

Miller, S. L.: Production of Some Organic Compounds Under Possible Primitive Earth Conditions. *J. Am. Chem. Soc.*, vol. 77, 1955, pp. 2351-2361.

Miller, Stanley L.; and Urey, Harold C.: Organic Compound Synthesis on the Primitive Earth. *Science*, vol. 130, no. 3370, July 31, 1959, pp. 245-251.

The Synthesis of Carbohydrates

Handbook of Chemistry and Physics, 43rd ed., pp. 10-13.

Akerlof, G. C.; and Mitchell, P. W.: Regeneration of Carbohydrates in a Closed Circuit Respiratory System. AIAA and Aerospace Medical Association Manned Space Laboratory Conference, Los Angeles, Calif., Rep. No. 63143, FMC Corp., May 1963.

Baeyer, Adolf: Ueber die Wasserentziehung und ihre Bedeutung für das Pflanzenleben und die Gährung. *Chem. Zentral.*, vol. 42, 1871, pp. 26-32.

Balezin, S. A.: The Mechanism of the Formation of Sugars from Formaldehyde. *J. Gen. Chem. USSR*, vol. 7, 1937, pp. 2099-2115 (C.A. 32: 503²).

Balezin, S. A.; and Surykina, E. K.: Chromatographic Method for Identifying the Condensation Products of Formaldehyde into Sugars. *Uchenye Zapiski Moskov. Gosudarst. Pedagog. Inst. im. V. I. Lenina*, vol. 99, Kafredra Obshchei i Anal. Khim., no. 6, 1957, pp. 159-165.

Betti, Mario; and Pratesi, Pretro: Optic Splitting of Racemic Glyceraldehyde. *Biochem. Z.*, vol. 274, 1964, pp. 1-3.

Binko, Ivan; and Kolář, Jaroslav: Trioses. Czech. Patent 92,198, Oct. 15, 1959 (C.A. 54: 15251d).

Breslow, R.: Mechanism of the Formose Reaction. *Tetrahedron Letters*, No. 21, 1959, pp. 22-26.

Cornubert, Raymond; and Peyrade, Jean: Étude des catalyseurs calciques de la saccharification d l'aldéhyde formique. *Compt. Rend.*, vol. 230, no. 6, Feb. 6, 1950, pp. 600-602.

Euler, H. V.; and Euler, A.: Ueber die Bildung von i-Arobinoketose aus Formaldehyd. *Ber.*, vol. 39, 1906, pp. 45-51.

Ewart, A. J.: The Synthesis of Sugars from Formaldehyde, Carbon Dioxide and Water. *Proc. Roy. Soc. Victoria*, vol. 31, no. 2, 1919, pp. 378-387; *Exptl. Sta. Record*, vol. 43, pp. 202-203.

Fischer, E.; and Passmore, E.: Bildung von Acrose aus Formaldehyd. *Ber.*, vol. 22, 1889, pp. 359-361.

- Fischer, E.; and Tafel, J.: Synthetische Versuche en der Zuckergruppe. 11. Ber., vol. 20, 1887, pp. 3384-3390.
- Fischer, H. O. L.; and Baer, Erich: Acetoneglyceraldehyde. 111. Synthesis of d-fructose and d-sorbose from d-glyceraldehyde or from d-glyceraldehyde and dihydroxyacetone. Helv. Chim. Acta, vol. 19, 1936, pp. 519-532.
- Fischer, H. O. L.; Taube, Carl; and Baer, Erich: Crystallized Glyceraldehyde and Its Conversion into Dihydroxyacetone. Ber., vol. 60B, 1927, pp. 479-485.
- Grubhofer, N.; and Schleith, L.: Die Spaltung von Racemischer Mandelsäure mit einem optisch aktiven Anionenaustauscher. Hoppe-Seylers Z. Physiol. Chem., vol. 296, 1954, pp. 262-266.
- Kirk, R.; and Othmer, D.; eds.: Encyclopedia of Chemical Technology. Vol. 4. Interscience, 1952, pp. 863-875.
- Kirk, R.; and Othmer, D., eds.: Encyclopedia of Chemical Technology. Vol. 5. Interscience, 1952, p. 97.
- Kirk, R.; and Othmer, D., eds.: Encyclopedia of Chemical Technology. Vol. 9. Interscience, 1952.
- Küster, William; and Schoder, Felix: Über das Entstehen von Sorbose bei der Kondensation des Formaldehyds. Hoppe-Seylers Z. Physiol. Chem., vol. 141, 1924, pp. 110-131.
- Kuzin, A.: Catalytic Effect of Monoses on the Condensation of Formaldehyde to Sugars. V. Course of Condensation Reaction in the Presence of Concentrated Salt Solutions. Biokhimiya, vol. 3, 1938, pp. 16-27 (C.A. 32: 4140⁶).
- Kuzin, A.: New Synthesis of Glycoaldehyde and Glyceraldehyde. J. Gen. Chem. USSR, vol. 8, 1938, pp. 592-595 (C.A. 33: 1271⁹).
- Landau, Bernard R.; and Merlevede, Wilfried: Initial Reactions in the Metabolism of D- and L-Glyceraldehyde by Rat Liver. J. Biol. Chem., vol. 238, 1963, pp. 861-867.
- Langenbeck, W.: Neue Untersuchungen über die Formaldehyd-kondensation. Angew. Chem., vol. 66, 1954, p. 151.
- Loew, O.: Weiteres über die Kondensation des Formaldehyds. J. Prakt. Chem., vol. 34, 1886, pp. 51-55.
- Loew, O.: Ueber einige katalytische Wirkungen. Ber., vol. 20, 1887, pp. 144-145.
- Loew, O.: Ueber Bildung von Zuckerarten aus Formaldehyd. Ber., vol. 22, 1887, pp. 470-478.

- Loew, O.: Zur Kondensation des Formaldehyds Berichlungung. Ber., vol. 39, 1906, pp. 1592-1593.
- Pfeil, E.; and Rucker, H.: Formaldehyde Condensation. Formation of Sugars from Formaldehyde by the Action of Alkalies. Ann., vol. 641, 1961, pp. 121-131.
- Pfeil, E.; and Schroth, G.: Kinetics and Reaction Mechanism of the Formaldehyde Condensation. Chem. Ber., vol. 85, 1952, pp. 293-307.
- Schmalfluss, H.: Studien über die Kondensation von Formaldehyd I: Über die Kondensation mit Magnesiumoxyd. Ber., vol. 57, 1924, pp. 2101-2104.
- Schmalfluss, H.: Über den Einfluss von Monosen und von Magnesiumion auf die Zuckerbildung aus Formaldehyd. Biochem. Z., vol. 185, 1927, pp. 70-85.
- Schmalfluss, H.; and Barthmeyer, H.: Über die Entstehung von Diacetyl aus Kohlenhydraten und verwandten Stoffen (Formation of Diacetyl from Carbohydrates and Related Substances). Ber., vol. 60, 1927, pp. 1035-1036.
- Seyewetz, A.; and Gibello: Synthésés de Sucres á Partir du Trioxyméthylíne et du Sulfite de Soude. Bull. Soc. Chim. Paris, vol. 31, series 3, 1904, pp. 434-440.
- Storch, Henry H.; Golumbic, Norma; and Anderson, Robert B.: The Fischer-Tropsch and Related Syntheses. John Wiley and Sons, New York, 1951.
- Von Butlerow, A. M.: Bildung einer Zuckerartigen Substanz durch Synthese. Ann., vol. 120, 1861, pp. 295-298.

Biological Systems: Gas Exchange

- Ammann, E. C. B.; and Lynch, V. H.: Gas Exchange of Algae. I. Effects of Time, Light Intensity and Spectral Energy Distribution of the Photosynthetic Quotient of Chlorella Pyrenoidosa. Appl. Microbiol., vol. 13, 1965, pp. 546-551.
- Bates, J. H.: Recent Aspects in the Development of a Closed Ecological System. Aerospace Med., vol. 32, 1961, pp. 12-24.
- Benedict, F. G.: A Portable Respiration Apparatus for Clinical Use. Boston Med. Surg. J., vol. 178, 1918, pp. 667-678.
- Benoit, R. J.: Mass Culture of Micro-Algae for Photosynthetic Gas Exchange. Algae and Man, D. F. Jackson, ed., Plenum Press, New York, 1964, pp. 413-425.
- Blasco, R. J.: Nature and Role of Bacterial Contaminants in Mass Cultures of Thermophilic Chlorella Pyrenoidosa. Appl. Microbiol., vol. 13, 1965, pp. 473-477.

- Bongers, L.: Chemosynthetic Gas Exchanger. Final Report, NASA Contract NASw-713, Martin Co., Denver, Colorado, 1964.
- Bongers, L.; and Kok, B.: Life Support Systems for Space Missions. Develop. Ind. Microbiol., vol. 5, 1964, pp. 183-195.
- Bovee, H. H.; Pilgrim, A. J.; Sun, L. S.; Schubert, J. E.; Eng, T. L.; and Beinishek, B. J.: Large Algal Systems. Biologistics for Space System Symposium, USAF Rep. AMRL-TDR-62-116, Wright-Patterson AFB, Ohio, 1962, pp. 8-19.
- Bowman, R. O.; and Thomae, F. W.: An Algal Life Support System. Aerospace Eng., vol. 19, 1960, pp. 26-30.
- Bowman, R. O.; and Thomae, F. W.: Long-Term Non-Toxic Support of Animal Life with Algae. Science, vol. 134, 1961, pp. 55-56.
- Canfield, J. H.; and Lechtman, M. D.: Study of Hydrogen-Fixing Microorganisms for Closed Regenerating Biosystems. USAF Rep. AMRL-TDR-64-35, Wright-Patterson AFB, Ohio, 1964.
- Consolazio, C. F.; Johnson, R. E.; and Becora, S. J.: Physiological Measurements of Metabolic Functions in Man. McGraw-Hill Book Co., Inc., New York, 1963, p. 53.
- Eley, J. H., Jr.; and Myers, J.: Study of Photosynthetic Gas Exchange. A Quantitative Repetition of the Priestley Experiment. Texas J. Sci., vol. 26, 1965, pp. 296-333; also USAF Rep. SAM-TDR-64-52, Brooks AFB, Texas.
- Foster, J. F.; and Litchfield, J. H.: A Continuous Culture Apparatus for the Microbial Utilization of Hydrogen Produced by Electrolysis of Water in Closed Cycle Space Systems. Biotech. Bioeng., vol. 4, 1964, pp. 441-456.
- Gafford, R. D.; and Bailey, R. V.: Flight Test of a Gravity-Independent Photosynthetic Gas Exchanger, A Feasibility Study. USAF Rep. SSD-TDR-63-240, Space Systems Division, Los Angeles, Calif., 1963.
- Gafford, R. D.; and Craft, C. E.: A Photosynthetic Gas Exchanger Capable of Providing for the Respiratory Requirements of Small Animals. USAF Rep. SAM-58-124, Brooks AFB, Texas, 1959.
- Golueke, C. G.; and Oswald, W. J.: Closing an Ecological System Consisting of a Mammal, Algae, and Non-Photosynthetic Organisms. Am. Biol. Teacher, vol. 25, 1963, pp. 522-528.
- Hannan, P. J.; and Patouillet, C.: Gas Exchange with Mass Cultures of Algae. I. Effects of Light Intensity and Rate of Carbon Dioxide Input on Oxygen Production. Appl. Microbiol., vol. 11, 1963, pp. 446-449.

- Korotayev, M. M.: Toxic Gaseous Substances Given Off by Chlorella. Vol. 3 of Problems of Space Biology, N. M. Siskayan and V. I. Yazdorskiy, eds., USSR Acad. Sci. Pub. House, Moscow; also Tech. Transl. 64-31578, U. S. Dept. Commerce, Joint Pub. Res. Service, Washington, D. C., 1964.
- Krall, A. R.; and Kok, B. : Studies on Algal Gas Exchangers with Reference to Space Flight. Develop. Ind. Microbiol. , vol. 1, 1960, pp . 33-44.
- London, S. A.; and West, A.: Gaseous Exchange in a Closed Ecological System. USAF Rep. AMRL-TDR-62-139, Wright-Patterson AFB, Ohio, 1962.
- Lynch, V. H.; Ammann, E. C. B.; and Godding, R. M.: Urine as a Nitrogen Source for Photosynthetic Gas Exchangers. Aerospace Med., vol. 35, 1964, pp . 1067-1071.
- Moss, D. N.: Plant Leaves for the Production of Oxygen in a Closed System. NASA CR-55131, 1963.
- Myers, J.: Study of a Photosynthetic Gas Exchanger as a Method of Providing for the Respiratory Requirements of the Human in a Sealed Cabin. USAF Rep. SAM 58-117, Randolph AFB, Texas, 1958, p. 8.
- Myers, Jack E.; and Brown, Allan H.: Gas Regeneration and Food Production in a Closed Ecological System. Natl. Acad. Sci., Natl. Res. Council Pub. 893, Washington, D. C., 1961.
- Nihei, L.; Sasa, S.; Miyachi, K.; Suzuki, K.; and Tamiya, H.: Change of Photosynthetic Activity of Chlorella Cells During the Course of Their Normal Life Cycle. Arch. Mikrobiol., vol. 21, 1954, pp. 156-166.
- Rossier, P. H.; Buhlmann, A. A.; and Wilsinger, K.: Respiration Physiologic Principles and Their Clinical Applications, P. C. Luchsinger and K. M. Moser, eds., C. V. Mosely Co., St. Louis, 1960, p. 113.
- Sorokin, C.: Changes in Photosynthetic Activity in the Course of Cell Development in Chlorella. Physiol. Plantarum, vol. 10, 1957, pp . 656-666.
- Ward, C. H.; Moyer, J. E.; and Vela, G. R.: Studies on Bacteria Associated with Chlorella Pyrenoidosa TX 71105 in Mass Culture. Develop. Ind. Microbiol., vol. 6, 1964, pp. 213-222; also USAF Rep. SAM-TDR-64-73, Brooks AFB, Texas.
- Ward, C. H.; Wilks, S. S.; and Craft, H. L.: Use of Algae and Other Plants in the Development of Life Support Systems. Am. Biol. Teacher, vol. 25, 1963, pp . 512-521.
- Wilks, S. S.: Preliminary Report on the Photosynthetic Gas Exchange Potentialities of the Family Lemnaceae (Duckweed). Biologistics for Space Systems Symposium, USAF Rep. AMRL-TDR-62-116, Wright-Patterson AFB, Ohio, 1962, pp . 265-278.

Wilks, S. S.: Biological Recycling Processes in Closed System. Lectures in Aerospace Medicine, USAF School of Aerospace Med., Aerospace Medical Division (AFSC), Brooks AFB, Texas, 1963, pp. 271-280.

Biological Systems: Food Production

Calloway, D. H.; and Margen, S.: Investigation of the Nutritional Properties of *Hydrogenomonas eutropha*. Semi-Annual Rep., NASA Grant NGR-05-003-089, Dept. Nutr. Sei., Univ. of Calif., Berkeley, 1965.

Fisher, A. W.; and Burlew, J. S.: Nutritional Value of Microscopic Algae. Algal Culture from Laboratory to Pilot Plant. Pub. 600, Carnegie Inst. of Washington, Washington, D. C., 1953, pp. 302-310.

Gorham, P. R.: Toxic Algae. Algae and Man, D. F. Jackson, ed., Plenum Press, New York, 1964, pp. 307-336.

Hayami, H.; and Shino, K.: Nutritional Studies on Decolourized *Chlorella*. Growth Experiment of Rats and the Digestibility of a Diet Containing 19 Percent of Decolourized *Chlorella*. Ann. Rep. Natl. Inst. Nutrition (Tokyo, Japan), 1958, pp. 56-58.

King, M. E.; and Shefner, A. M.: Enzymatic Digestion of Algal Cells. USAF Rep. AMRL-TDR-62-91, Wright-Patterson AFB, Ohio, 1962.

Leveille, G. A.; Sauberlick, H. E.; and Edelbrock, J. A.: The Influence of Enzyme Supplementation of the Digestibility of Algae. Rep. No. 259, U. S. Army Med. Res. Nutr. Lab., Fitzsimmons General Hospital, Denver 30, Colorado, 1961.

Lubitz, J. A.: The Protein Quality, Digestibility, and Composition of *Chlorella* 71105. ASD Tech. Rep. 61-535, Wright-Patterson AFB, Ohio, 1961.

McDowell, M. E.; and Leveille, G. A.: Feeding Experiments with Algae. U. S. Army Med. Res. and Nutr. Lab., Fitzsimmons General Hospital, Denver 30, Colorado, 1964.

Meffert, M. E.; and Pabst, W.: Über die Verwert-Barkeit der Substanz von *Scenedesmus Obliquus* als Eiweißquelle in Ratten-Bilanz-Versuchen. Nutr. Dieta, vol. 5, 1963, pp. 235-254.

Milner, H. W.: The Chemical Composition of Algae. Algal Culture: From Laboratory to Pilot Plant, J. S. Burlew, ed., Pub. 600, Carnegie Inst. of Washington, Washington, D. C., 1953, pp. 285-302.

Moore, B. G.; and Tischer, R. G.: Extracellular Polysaccharides of Algae: Effects on Life Support Systems. Science, vol. 145, 1964, p. 586.

Morimura, Y.; and Tamiya, N.: Preliminary Experiments in the Use of *Chlorella* as Human Food. Food Technol., 1954, pp. 179-182.

- Oswald, W. J.; Golueke, C. G.; Cooper, R. C.; Myers, J. H.; Hintz, H.; and Gee, H. K.: Nutritional and Disease Transmitting Potential of Sewage-Grown Algae. First Progress Rep. WP 00026 R1-R2-R3, SERL Rep. 64-6, Sanitary Engineering Res. Lab., Univ. of Calif., Berkeley, 1964.
- Pabst, W. F.; Jekat, F.; and Rolle, I.: The Utilization of Carbohydrates, Fats, Phosphorous and Nitrogen from Roller-Dried Schenedesmus Substance as Shown by Balance Studies in the Rat. Nutr. Dieta, vol. 6, 1964, pp. 279-295.
- Powell, R. C.; Nevels, E. M.; and McDowell, M. E.: Algae Feeding in Humans. J. Nutr., vol. 75, 1961, pp. 7-12.
- Tamiya, H.: Chemical Composition and Applicability as Food and Feed of Mass-Cultured Unicellular Algae. Final Rep. No. 1, TOKU 33129 FR-1, U. S. Army Res. and Develop. Group, 1961.
- Tischer, R. B.; and Moore, B. G.: An Extracellular Polysaccharide Produced by *Palmella mucos.* Arch. Mikrobiol., vol. 49, 1964, pp. 158-166.
- Wilks, Syrrrel S.: Plant Systems as Long Term Flight Nourishment Sources. Conference on Nutrition in Space and Related Waste Problems. NASA SP-70, 1964, pp. 305-310.

Biological Systems: Waste Disposal

- Combustion of Human Waste and Product Recovery. TN 62-16, Arctic Aeromedical Lab., Air Force Systems Command, Fort Wainwright, Alaska, 1963.
- Goldner, B. H.: Application of Hydrogen Bacteria to Bioregenerative Life Support Systems. I. Urine in *H. eutropha* Cultures. Rep. No. NASw-988, Magna Corp., Anaheim, Calif., 1965.
- Golueke, C. G.; Oswald, W. J.; and McGauhey, P. H.: The Biological Control of Enclosed Environments. Sewage Ind. Wastes, vol. 31, 1959, p. 1125.
- Oswald, W. J.; and Golueke, C. G.: Microbiological Waste Conversion in Control of Isolated Environments. AF 19(604)-6637, School of Sanitary Engineering, Univ. of Calif., Berkeley, 1960.
- Pipes, W. O.: Waste-Recovery Processes for a Closed Ecological System. Pub. 898, Natl. Acad. Sci., Natl. Res. Council, Washington, D. C., 1961.
- Pote, H. L.: Waste Conversion by Anaerobic Thermophilic Methods. Biologistics for Space Systems Symposium, USAF Rep. AMRL-TDR-62-116, Wright-Patterson AFB, Ohio, 1962.
- Tischer, R. G.; Brown, L. R.; and Kennedy, M. V.: Physical, Chemical and Microbiological Evaluation of Electrochemical Degradation of Human Wastes. Final Rep. AF41609 1633, Mississippi State Univ., 1964.

Less Studied Candidates for Microbial Life Support Systems

- Barker, H. A.: Fermentation of Nitrogenous Organic Compounds. The Bacteria. Vol. II of Metabolism, I. C. Gunsalus and R. Y. Stanier, eds., Academic Press, New York, 1961, pp. 151-207.
- Breed, Robert S.; Murray, E. G. D.; and Smith, Nathan R., eds.: Society of American Bacteriologists. Bergey's Manual of Determinative Bacteriology. Seventh ed., Williams and Wilkins Co., Baltimore, 1957.
- Brown, L. R.; and Strawinski, R. J.: The Bacterial Metabolism of Methane. Bacteriol. Proc., 1957, pp. 18-19.
- Brown, L. R.; and Strawinski, R. J.: Intermediates in the Oxidation of Methane. Bacteriol. Proc., 1958, p. 22.
- Burton, R. M.; and Kaplan, N. O.: A DN Specific Glycerol Dehydrogenase from Aerobacter aerogenes. J. Am. Chem. Soc., vol. 75, 1953, pp. 1005-1006.
- Chaix, P.; Luthiois, A.; and Issoly, I.: Vitesse de respiration de B. subtilis dans son milieu de culture: Influence de CO₂. Biochim. Biophys. Acta, vol. 29, 1958, pp. 151-160.
- Champagnet, A.: Protein from Petroleum. Scientific American, vol. 213, 1965, pp. 13-17.
- Cheldelin, V. H.: Metabolic Pathways in Microorganisms. E. R. Squibb Lectures on Chemistry of Microbial Products. John Wiley and Sons, New York, 1961.
- Clifton, C. E.: Microbial Assimilations. Advan. Enzymol., vol. VI, 1946, pp. 269-308.
- Davis, J. B.; Coty, V. F.; and Stanley, J. P.: Atmospheric Nitrogen Fixation by Methane-Oxidizing Bacteria. J. Bacteriol., vol. 88, 1964, pp. 468-472.
- DeLey, J.; and Schell, J.: The Oxidation of Several Substrates by Acetobacter aceti. J. Bacteriol., vol. 77, 1959, pp. 445-451.
- Dolin, M. I.: Survey of Microbial Electron Transport Mechanisms. The Bacteria. Vol. II of Metabolism, I. C. Gunsalus and R. Y. Stanier, eds., Academic Press, New York, 1961.
- Dworkin, M.; and Foster, J. W.: Studies on Pseudomonas Methanica (Sohngen) Nov. Comb. J. Bacteriol., vol. 72, 1956, pp. 646-659.
- Elsden, S. R.: Photosynthesis and Lithotrophic Carbon Dioxide Fixation. The Bacteria. Vol. III of Biosynthesis, I. C. Gunsalus and R. Y. Stanier, eds., Academic Press, New York, 1962, pp. 1-40.
- Foster, J. W.: Chemical Activities of the Fungi. Academic Press, New York, 1949.

- Fuhs, G. W.: Der Mikrobielle Abbau von Kohlenwasserstoffen. Arch. Mikrobiol., vol. 39, 1961, pp. 374-422.
- Gunsalus, I. C.; Horecker, B. L.; and Wood, W. A.: Pathways of Carbohydrate Metabolism. Bacteriol. Rev., vol. 19, 1955, pp. 79-128.
- Gunsalus, I. C.; and Umbreit, W. W.: The Oxidation of Glycerol by Streptococcus Faecalis. J. Bacteriol., vol. 49, 1945, pp. 347-357.
- Hers, H. G.: Triokinases. The Enzymes, P. D. Boyer, H. Lordy, and K. Myrback, eds., vol. 6, Academic Press, New York, 1962, pp. 75-83.
- Huennekens, F. M.; and Osborn, M. J.: Folic Acid Coenzymes and One Carbon Metabolism. Advan. Enzymol., vol. 21, 1959, pp. 369-446.
- Hutton, W. E.; and Zobell, C. E.: The Occurrence and Characteristics of Methane-Oxidizing Bacteria in Marine Sediments. J. Bacteriol., vol. 58, 1949, pp. 463-473.
- Jacobs, N. J.; and Van De Mark, P. J.: Comparison of the Mechanism of Glycerol Oxidation in Aerobically and Anaerobically Grown Streptococcus Faecalis. J. Bacteriol., vol. 79, 1960, pp. 532-538.
- Kaserer, H.: Die Oxydation des Wasserstoffes durch Mikroorganismen. Zbl. Bact. Abt. 11, vol. 16, 1906, p. 681.
- Klungsoyr, L.; King, T. E.; and Cheldelin, V. H.: Oxidative Phosphorylation in Acetobacter Suboxydans. J. Biol. Chem., vol. 227, 1957, pp. 135-149.
- Kluyver, A. J.; and Sehnellin, G. T. P.: On the Fermentation of Carbon Monoxide by Pure Cultures of Methane Bacteria. Arch. Biochem., vol. 14, 1947, pp. 57-70.
- Maurer, P. R.; and Batt, R. D.: Oxidation of Polyols by Nocardia Corallina. J. Bacteriol., vol. 83, 1962, pp. 1131-1139.
- McClung, N. M.: The Utilization of Carbon Compounds by Nocardia Species. J. Bacteriol., vol. 68, 1954, pp. 231-236.
- McCulloch, E. C.: Disinfection and Sterilization. Lea and Febiger, Philadelphia, 1945.
- McIntosh, E. N.; Purko, M.; and Wood, W. A.: Ketopantoate Formation by Hydroxymethylation Enzyme from Escherichia coli. J. Biol. Chem., vol. 228, 1957, pp. 499-510.
- McKenna, E. J.; and Kallio, R. E.: The Biology of Hydrocarbons. Ann. Rev. Microbiol., vol. 19, 1965, pp. 183-208.
- Nechaeva, N. B.: Two Species of Methane Oxidizing Mycobacteria. Mikrobiologiya, vol. 18, 1949, pp. 310-317.

- Orla-Jensen, S. : Die Hauptlinien des Natürlichen Bakteriensystems . Zentr .
Bakteriol. Parasitenk., Abt. II, vol. 22, 1909, pp. 305-346.
- Purko, M.; Nelson, W. O.; and Wood, W. A.: The Role of p-Aminobenzoate in
Pantoate Synthesis by Bacterium Linens. J. Biol. Chem., vol. 207, 1954,
pp. 51-58.
- Quayle, J. R.: Metabolism of C₁ Compounds in Autotrophic and Heterotrophic
Microorganisms. Ann. Rev. Microbiol ., vol . 15, 1961.
- Rush, D.; Karibian, D.; Karnovsky, M. L.; and Magasanik, B. : Pathways of
Glycerol Dissimilation in Two Strains of Aerobacter aerogenes: Enzymatic
and Tracer Studies. J. Biol. Chem., vol. 226, 1957, p. 891.
- Smith, L. : Cytochrome Systems in Aerobic Electron Transport. The Bacteria.
Vol. II of Metabolism, I. C. Gunsalus and R. Y. Stanier, eds., Academic
Press, New York, 1961.
- Sobolov, M.; and Smiley, K. L.: Metabolism of Glycerol by an Acrolein-Forming
Lactobacillus. J. Bacteriol., vol. 79, 1960, pp. 261-266.
- Sohngen, N. I.: Ueber Bakterien welche Methan als Kohlenstoffnahrung und
Energiequelle gebrzuchen. Zentr . Bakteriol . Parasitenk ., Abt . II, vol . 15,
1906, pp. 513-517.
- Utter, M. F.; and Wood, H. G.: Mechanisms of Fixation of Carbon Dioxide by
Heterotrophs and Autotrophs. Advan. Enzymol. , vol. XII, 1952, pp. 41-151.
- Virtanen, A. I.; and V. Hausen, J.: Uber die Vergarung von Glyceraldehyd.
Z. Physiol. Chem., vol. 204, 1932.
- Wehmer, C. : Biochemische Zersetzung des Kohlenoxyds . Ber . Deut. Chem. Ges .,
vol. 59, 1926, p. 887.
- Wiame, J. M.; Bourgeois, S.; and Lambion, R. : Oxidative Dissimilation of
Glycerol Studied with Variants of Bacillus subtilis. Nature, vol. 174,
1954, pp. 37-38.
- Wood, W. A.: Fermentation of Carbohydrates and Related Compounds. The
Bacteria. Vol. II of Metabolism, I. C. Gunsalus and R. Y. Stanier, eds.,
Academic Press, New York, 1961, pp. 59-149.
- Zobell, C. E. : Assimilation of Hydrocarbons by Microorganisms. Advan.
Enzymol., vol. X, 1950, pp. 443-486.

Algae Lighting

- Novosad, R. S.; and Dezur, R. S.: An Analysis of a Fiber Method of Illumina-
tion for an Algae Suspension in a Life Support System. AAS Preprint 63-22,
1963.

Algae Systems Engineering

Golueke, C. G.; Oswald, W. J.; and Lee, H. K.: A Study of Fundamental Factors Pertinent to Microbiological Waste Conversion in Control of Isolated Environments. First Tech. Rep. AFCRL-64-341, March 31, 1964.

PLANS FOR A PROGRAM TO STUDY CLOSED
LIFE-SUPPORT SYSTEMS

By E. Gene Lyman

Ames Research Center

Ames Research Center has a program to study closed life-support systems. The first phase includes the General Dynamics/Convair and Lockheed Missiles and Space Company studies that led to the definition of configurations of closed systems. The purpose of this presentation is to describe the plan which the Ames Research Center hopes to follow in pursuing a closed life-support system program.

Ames' overall objective is to conduct research and development on advanced life-support systems to establish a foundation of technology for assuring the safety and support of man in long duration space missions. The development program for a closed life-support system is an important contribution to this overall objective. It should be noted that this program is not specifically identified with any future NASA missions, but only endeavors to provide quantitative information for planning future missions. However, consideration must be given to the type of missions in which a closed system could be used because of (1) the need to assess the influence of such obvious factors as crew size, mission time duration, operational modes, power systems, etc., on the selection of a workable life-support system configuration and (2) the need to assess the degree to which resources should be committed to the support of the program.

The question is whether we can reasonably predict the requirement for a closed system with sufficient accuracy to determine the degree to which resources should be devoted to its development. Currently, a great deal of attention is being given to defining NASA's mission beyond the various Apollo programs. There have been recommendations that the next family of manned missions be devoted to planetary exploration. Various mission analyses have shown that approximately 400 to 1,000 days will be required to accomplish flyby missions to such planets as Venus, Mars, and Jupiter. Similar studies have also shown crew requirements on the order of 10 to 20 men. The results of the studies presented in preceding papers have shown that in 4,000 to 10,000 man-day missions a closed life-support system becomes competitive, and this magnitude of mission falls within the scope of planetary missions.

However, these competitive estimates were based on assumptions not entirely quantitative. For example, how does one determine the relative ratings of a system based on factors such as maintainability, reliability, and safety, particularly when a life-support system of comparable complexity has yet to be used in a flight mission? In fact, the flight qualification of a system an order of magnitude less complex than a food synthesis system has yet to be made. Prior studies of missions of intermediate time duration have

shown that the ability to recover potable water from urine and oxygen from carbon dioxide will offer great savings in weight, volume, and power requirements over open systems that carry expendable stores of oxygen and water.

Yet, has an acceptable water electrolysis system or an acceptable water recovery system been developed? Sufficient research and development has been accomplished on such systems as water electrolysis and CO₂ reduction so that flight systems could be constructed and evaluated; the Air Force and NASA have such programs under way. But, in spite of the various approaches that have been pursued on recovering water from urine, no one system is being actively developed for flight evaluation. The reason may be that no specific missions have been planned that require water recovery; but water recovery is a relatively complex process that requires considerable pretreatment and post-treatment, separation of products, and operation with materials in various phases, that is, gas, liquid, and solid.

Consider a representative process that may be encountered in a food Synthesis system. For this discussion, a formaldehyde synthesis system is shown in figure 1. This particular technique is based on the conversion of

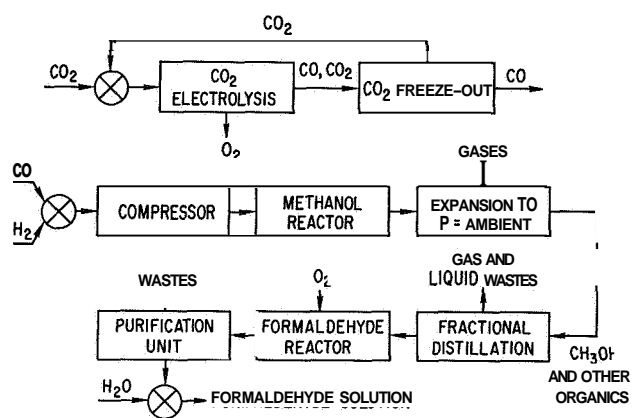


Figure 1.- Block diagram of representative formaldehyde synthesis process.

apparent that none of these processes has been accomplished in qualified life support systems.

From study of the formaldehyde synthesis process, it appears that a great portion of the closed life-support system program will consist in determining techniques for the application of conventional chemical processes to the spacecraft system. This application will not be simple, as evidenced by the separation problems encountered in water recovery systems for example. Other problems that will be encountered have yet to be solved practically in the Earth environment, as for example, the resolution of racemic mixtures of sugars.

Having considered the magnitude of the problems that confront us, it is now appropriate to consider what course to pursue in achieving a quantitative closed life-support system technology. The preceding papers have made it abundantly clear that a great deal of work needs to be done. We have outlined

a plan for conducting this program that we believe will accomplish our objectives in a minimum time as well as make maximum use of our limited available resources.

The first task was to identify critical areas that would have a "go-no-go" impact on the program. Then, tasks that required advanced technology to quantify their feasibility were identified; and finally, tasks were identified which required the investigation or determination of new concepts.

The most important question for any food synthesis concept is whether or not the food is nutritionally adequate. To answer this question, a program for the initial screening of potential foodstuffs has been established and is now under way. In this program, the nutritional suitability of such materials as glycerol, glyceraldehyde, dihydroxyacetone, and formose sugar mixtures will be studied on small animals. Materials fed in these studies will be furnished in the pure form as well as in the same form as they would be obtained in the actual synthesis process. Early indications of acceptability of the food will allow the programs to proceed efficiently without unwarranted attention to unpromising concepts. In line with the nutritional suitability of a foodstuff, immediate attention will be given to producing sufficient quantities of Hydrogenomonas eutropha bacteria for feeding studies. As stated earlier, only preliminary short-term feeding studies have been undertaken with this bacteria and it is essential to obtain more quantitative data on them before extensive effort is devoted to developing a representative system. The question raised by Lockheed with respect to the feeding of high protein diets must be answered. The nutritional problem then may be classified as a "go-no-go" situation and, as such, candidate foodstuffs will be screened as soon as possible. Although we consider the nutritional aspects of the program predominant in their eventual impact on a particular food synthesis system, we are reasonably confident on the basis of available information that the materials produced in the various systems described today will be nutritionally suitable. Therefore, we will proceed directly to the development of certain processes to the breadboard level. Formaldehyde synthesis may be approached in this manner. Formaldehyde is produced commercially in great quantities, and the reaction conditions and the processes are well established. The major effort in this program will be to develop new techniques that may be used in the spacecraft environment (e.g., separating formaldehyde from a methanol-formaldehyde solution).

The production of sugars from formaldehyde is not so simple. Many approaches will have to be investigated in order to solve this problem satisfactorily. We intend to study both long chain sugars from the formose mixture and the production of short chain materials, such as glyceraldehyde, glycerol, and dihydroxyacetone. Techniques for separating unusual products will have to be developed. There are no commercially available processes for separating racemic mixtures of sugars and this may be a major problem.

In addition to providing adequate quantities of Hydrogenomonas for feeding experiments, the environmental conditions under which Hydrogenomonas growth may be sustained will be more fully defined. Particular attention will be given to determining the effects of long-term operation of continuous cultures, and eventually to culture operation with the nutrient medium

recycled. This work will also require suitable separation techniques. It will be necessary to determine the nutritive value of the culture medium obtained from urine and feces mixtures as well as the role of nutrients in feces in a closed system.

In line with the analytical studies on feces, further analysis will be made of the closed life-support system. In the papers presented here various constraints were imposed and assumption were made. We will reexamine the closed system configuration in light of altered constraints and assumptions. For example, we will consider a closed life-support system that will have available sunlight, or one which would be used on a planetary surface or in a reduced gravity field. The feasibility of using metabolic wastes as possible fuels or propellants will be assessed. In these studies, new and old work will be reviewed continually in an attempt to assure that the most feasible concepts are being considered for a closed life-support system.

Briefly I have just described the tasks we intend to pursue so that within three years we will have sufficient technology available to quantify some of the information presented today. To this end we hope to have established and developed breadboard configurations of certain key concepts that will be required in a first generation closed life-support system.

One may well ask what course will be followed if there is a major failure or lack of success in any one of our given tasks. Our intention is to investigate other areas not yet defined. These may be categorized as exploratory; and our intention is to devote about 25 percent of our resources to the study of such concepts.

The discussion of the research tasks we are undertaking has been brief thus far. We have not specified the resources to be devoted to any given task or the specific schedule to be followed in accomplishing these tasks. This omission has been intentional for several reasons. The major reasons are that we can only estimate on a yearly basis the resources available and that we cannot estimate the success of any given task.

The continuation of Phase I studies will be analytical and will consider such factors as operation in a reduced gravity field, recovery of fuels or propellants from metabolic wastes, etc.

A small contract has been awarded for the initial evaluation and screening of potential synthetic foodstuffs. Planning is still under way for the larger scale nutrition program that must be conducted to support this program. It is hoped that feeding studies utilizing man will begin in approximately two years.

One continuous culture Hydrogenomonas system will be built. It will be used for investigating various environmental factors that influence the operation of the culture. Tentatively, we are planning to investigate the problems associated with applying a Hydrogenomonas culture to a spacecraft system. In a chemical synthesis of formaldehyde the techniques for forming formaldehyde from CO, and H₂ will be determined.

It is appropriate at this time to mention the overall program and the time scale in which we hope to accomplish various phases of the program. The Phase II portion of the program concerns the research and development of specific subsystems. We are just now entering this phase and descriptions have been given of the various tasks we hope to undertake. We hope to achieve a reasonable level of technology within three years for various key subsystems that will permit the engineering design of a first generation closed system.

We have already undertaken the procurement of systems for use in conjunction with a closed life-support system. To this end, a large environmental chamber will be installed at Ames by April 1967. Physicochemical systems for such functions as removing CO₂, recovering oxygen, and controlling temperature and humidity will be installed. Preliminary confinement studies and habitability studies would be conducted in this chamber until we may begin studies with the closed life-support system.

The objective of our research programs and the factors which influenced our decisions for conducting a closed life-support system program have been briefly described as have the research areas we hope to undertake.

WASTE STABILIZATION IN SPACE ENVIRONMENTS

By H. G. Schwartz, Jr., and J. E. McKee

W. M. Keck Laboratory of Environmental Health Engineering
California Institute of Technology

One of the major problems to be solved before extended space voyages can be realistically planned and programmed is that of waste management and water reuse. The logistics of space travel necessitate the reclamation of water from urine, wash water, condensate water, and possibly feces. To obtain a safe potable supply from such used waters, it is necessary to remove chemical and biological contaminants.

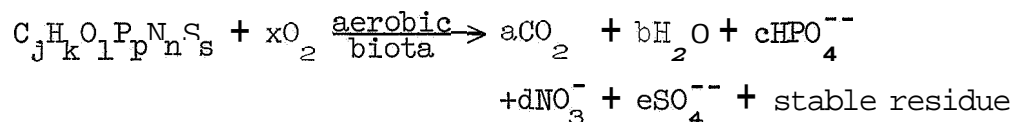
The principal purpose of this paper is to discuss one particular means of biological waste stabilization of possible value in an integrated system for waste and water management in space. Before doing so, however, it might be well to bring the reader into rapport with some of the alternative methods of waste-water purification. This subject has been dealt with by others (refs. 1-3), but a brief review of the methods proposed for water recovery and waste stabilization is appropriate.

WATER RECOVERY SYSTEMS

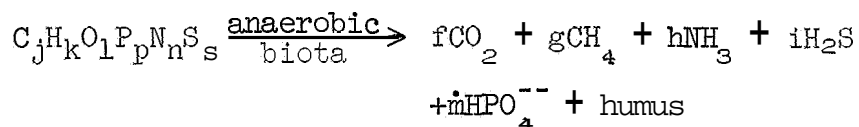
Several physical and chemical systems for water reclamation have been studied by other investigators, including: (a) distillation, (b) freeze drying, (c) electrodialysis, (d) ion-exchange processes, (e) activated carbon adsorption, (f) membrane permeation, (g) vapor pyrolysis, (h) combustion, and (i) electrolysis. To date, it appears that none of these systems operating independently is wholly satisfactory, although combinations show promise. Organic carryover in distillation and freezing operations necessitates additional treatment by activated carbon adsorption. Electrodialysis and ion-exchange techniques will not remove most of the nonpolar organic contaminants. Adsorption on activated carbon will remove most of the organic matter, but a system for efficiently regenerating the exhausted carbon has not been developed. Fouling of the membranes presents difficulties in the membrane permeation technique. The high temperatures required for vapor pyrolysis and combustion processes offer some disadvantages. The electrolysis-fuel-cell arrangement, although appealing in its fundamental concepts, has a high power requirement and produces some organic contamination of the product water.

It is apparent that, in one way or another, the organic matter in the wastes presents great difficulties for many of the physical and chemical processes. Based on terrestrial experience, it might be appropriate to consider biological processes for stabilization and mineralization of the organic substances prior to physical or chemical processes for demineralization.

An organic waste may be subjected to biodegradation by either aerobic or anaerobic processes. Some fundamental differences between aerobic and anaerobic stabilization should be examined in the light of their use in space environments. The final products from the aerobic decomposition of organic matter are carbon dioxide, water, nitrates, phosphates, sulfates, and similar well-oxidized compounds, thus:



Anaerobic degradation yields ammonia, humus, carbon dioxide, water, methane, and sulfides, thus:



In a closed environment, the carbon dioxide, nitrates, sulfates, etc., from aerobic stabilization would be innocuous end products. On the other hand, ammonia, humus, methane, and sulfides (notably hydrogen sulfide) would present new control problems. Aerobic systems, therefore, are inherently better suited for space environments.

Several aerobic processes are presently used to treat municipal and industrial wastes. Among the more common systems that have been investigated by various contractors for use in space vehicles are: (a) activated sludge, (b) trickling filters, and (c) oxidation ponds. The absence of gravity imposes severe limitations on these processes. The separation of liquid and solid components in the effluents from the activated-sludge and oxidation-pond processes is difficult and expensive with artificial gravity. To trickle waste over a coarse filter would also require an induced gravitational field. While the problems encountered with these systems do not and should not eliminate them from consideration, their application may be limited.

FILTRATION THROUGH FINE POROUS MEDIA

To overcome some of the difficulties presented by gravity-free conditions, McKee (ref. 4) proposed the use of two-phase aerobic percolation through fine porous media to stabilize organic waste waters in space environments. Intermittent filtration of municipal wastes through beds of sand has been used for many years as a secondary treatment process. The effluent from such a process is extremely stable as evidenced by 95 to 99 percent reductions in the five-day biochemical oxygen demand (BOD) and a high degree of nitrification. Intermittent sand filtration generally yields an effluent superior to that from typical activated-sludge and trickling-filter processes. The relatively large land areas required for sand filtration, however, have limited its use

for municipal wastes. Recently, this system was studied for use as a tertiary polishing treatment following the activated sludge process in conjunction with the recharging of depleted ground-water storage (ref. 5).

In view of the highly stabilized effluent obtainable from intermittent sand filtration, this process should be considered for biodegradation of the organic constituents of waste waters in space vehicles. Furthermore, since the capillary forces so important in this system do not depend on gravity, the system should operate in a zero-gravity environment with no difficulty. Interest at the California Institute of Technology in the waste treatment problems encountered in space environments has led to a study of intermittent sand filtration as a possible means of biodegradation of urine and other organic waste water. This project is being sponsored by NASA through the Ames Research Center.

EXPERIMENTAL EQUIPMENT

A schematic diagram of the sand columns presently being employed on this study is shown in figure 1. The columns are made of 1-inch-diameter lucite

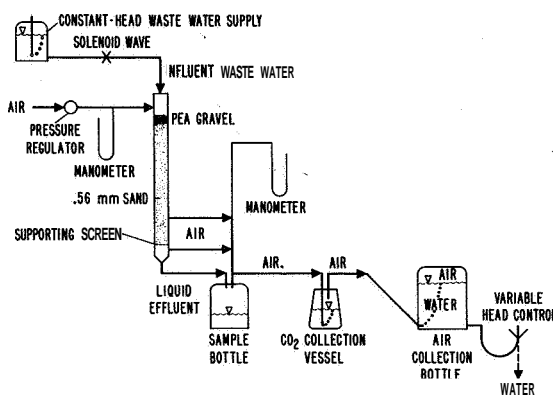


Figure 1.- Schematic diagram of intermittent sand filtration apparatus.

approximately 30 inches in length. A wire screen near the bottom of each column supports about 24 inches of sand. Above this fine porous medium, a 1-inch layer of pea gravel distributes the intermittent dosage and prevents the formation of scum.

The waste is applied at regular intervals to the top of the sand by a solenoid valve-time switch arrangement. Both the amount of waste applied per dose and the frequency of dosing can be easily adjusted. Liquid samples are collected at the bottom of the columns.

Air or oxygen is supplied above the column of sand. The air passing through the sand is put through a CO₂ collector and the remaining volume is then measured. Gas pressures on both the influent and effluent sides can be regulated to control the gas flow. A photograph of the laboratory apparatus appears in figure 2.

Two sizes of sand are being used in the experimental work. Most of the columns contain sand with a geometric mean size of 0.56 mm and a geometric standard deviation of 1.2. Several columns were prepared with a finer sand with a geometric mean size of 0.12 mm and a geometric standard deviation of 1.14. Very low infiltration rates and excessive ponding were encountered with the finer sand.

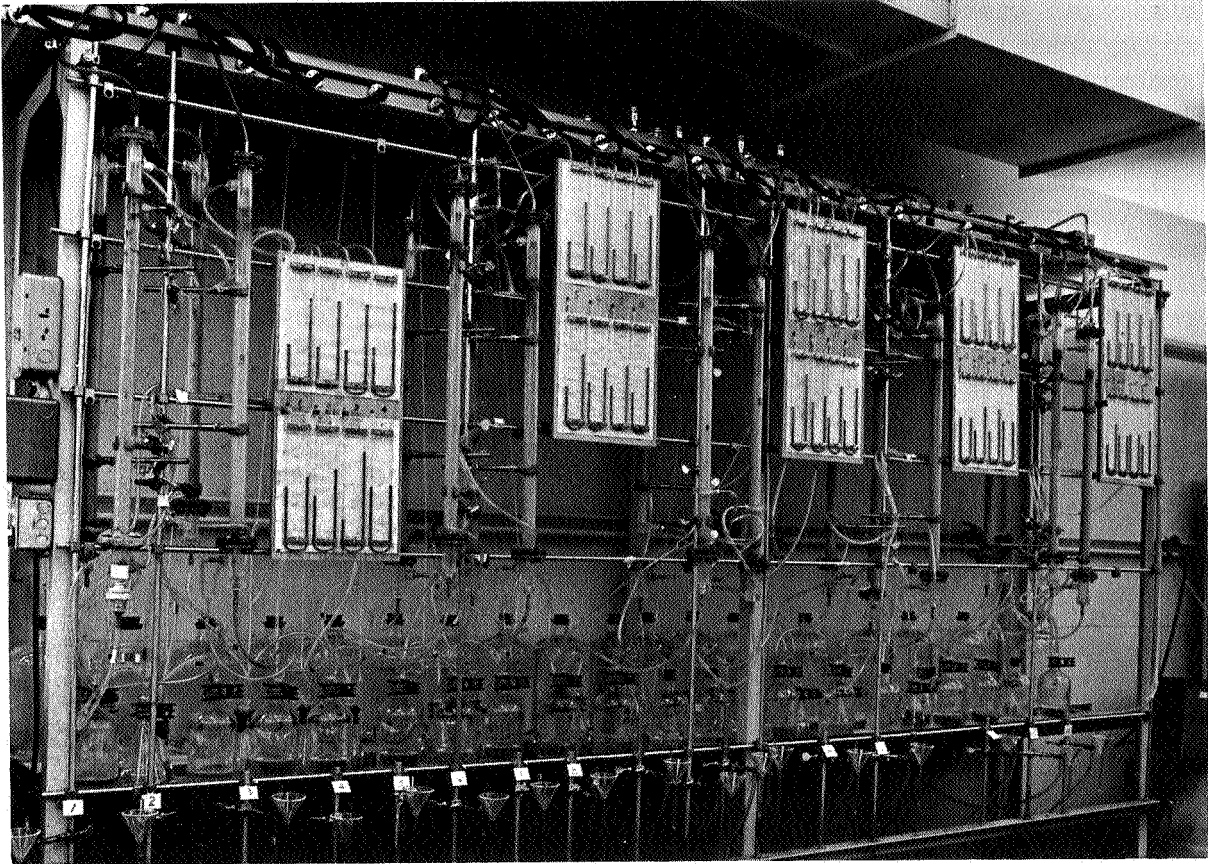


Figure 2.- View of intermittent sand filters.

PROCEDURE AND RESULTS

In an intermittent sand filter, as with other biological processes, it is necessary to develop an abundant microbial culture capable of degrading the organic constituents in the waste feed. To obtain such a population, settled sewage from the Whittier Narrows Water Reclamation Plant has been used. The effluent from the primary sedimentation tank at that plant is filtered through glass wool and then fed intermittently to the sand columns.

The first column was placed in operation in August 1965. Since then, this column has been dosed with settled sewage at the rate of 100-150 ml/day or 20-30 cm/day. Almost immediately, the chemical oxygen demand (COD) decreased from about 200-250 mg/liter in the applied sewage to 30-40 mg/liter in the effluent as shown in figure 3. It is known from the Whittier Narrows study (ref. 5) that removal of COD occurs largely as a result of physical adsorption.

Complete acclimatization in the experimental columns, however, was not evidenced for about 40 days. At that time, the nitrates in the effluent increased dramatically with a concomitant decrease in the organic and ammonia nitrogen content. The behavior of this system with respect to the effluent nitrogenous material is shown in figure 4.

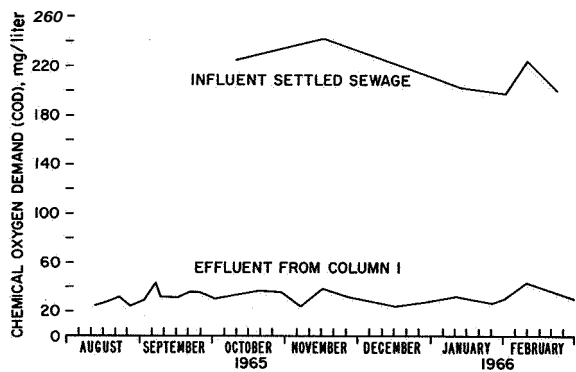


Figure 3.- Chemical oxygen demand of influent and effluent for column 1.

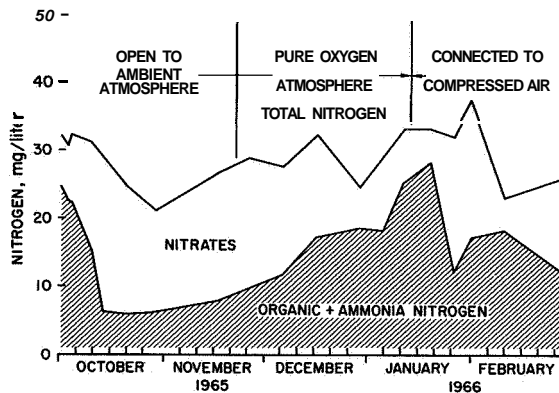


Figure 4.- Nitrogen content of effluent from column 1.

In November 1965, this column was connected to a pure oxygen supply. Prior to this time, the system had been open to the atmosphere. It was hoped that the oxygen atmosphere would be conducive to further nitrification. That the opposite effect was observed is apparent in figure 4. Nitrification decreased markedly during the 59 days the system was exposed to pure oxygen. A review of soil-science literature indicated that high oxygen tensions appear to have a detrimental effect on nitrifying microorganisms. The oxygen supply was removed in January 1966 and the column was connected to a compressed-air line. Since that time, the column has shown an improved ability to convert organic and ammonia nitrogen into nitrates.

The sporadic behavior of this sand filter during the past few months has also been attributed to difficulties encountered with the airflow through the column. It is evident that capillary forces form a saturated zone above the supporting screen which prevents airflow. Modifications in the effluent air-collection arrangement have eliminated this problem. With continuous or semi-continuous airflow, a highly nitrified effluent is obtained.

Fifteen additional columns were put into operation in October 1965. Seven of these columns contained 0.56 mm sand while the remainder held 0.12 mm sand. The coarse sand filters required from 50 to 100 days before measurable nitrification was observed.

Ponding occurred on all of the fine-sand filters shortly after dosing began. Infiltration rates were so low that these systems had to be eliminated from practical consideration. Moreover, significant nitrification was demonstrated only after 146 days.

All but two of the fine filters, therefore, were emptied in January 1966 and refilled with the coarser medium. In an effort to develop these new Systems more rapidly, sand from a previously acclimated filter was placed on top of the new sand columns. Some nitrification was observed immediately and a high degree of nitrification was obtained within 5 to 25 days. Figure 5 shows the results with one such column.

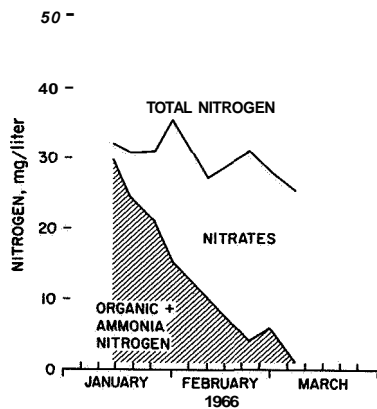


Figure 5.- Nitrogen content of effluent from seeded column 15.

The results of the research to date are somewhat limited with respect to the eventual impact of the study on space travel, inasmuch as the project has been under way for less than one year. It is apparent that the acclimation of intermittent sand filters can be accomplished rapidly using inoculum from other biologically active filters. In space vehicles, a spare sand filter could possibly be activated within a very short period using a frozen microbial population from a system acclimated on earth.

DISCUSSION

The total waste-water output from one man has been calculated to be about 2500 ml/day (ref. 6). Additional water requirements for bathing and laundry have been estimated by Ingram (ref. 2) to be 5.5 to 13.5 liters/man/day giving a total of 8.0 to 15.0 liters/man/day. On the basis of the research on intermittent sand filters, 10.0 liters/day of settled municipal sewage could be treated with a column 20.6 cm in diameter at the dose rate of 30 cm/day currently being employed. It is anticipated that the present dose rate can be increased considerably with no adverse effects on the effluent quality. McMichael and McKee (ref. 5) recently completed a three-year study on the intermittent percolation of secondary effluent through soil beds. A high quality effluent was obtained at percolation rates as high as 158 cm/day. This information leads to the conclusion that, after sufficient acclimatization, the dosage rates with settled sewage may be raised four to five times their present value. For purposes of this discussion, it will be assumed that the rate of application for settled sewage can be raised to 100 cm/day. At this rate, only 100 cm² or a column 11.3 cm in diameter would be necessary to treat 10.0 liters/day of settled municipal sewage.

Before this information obtained from the experimental work can be extrapolated to the waste waters in space environments, the strengths of the wastes must be compared. The average components of urine are given in table I, after McKee (ref. 4). From these data, an average theoretical oxygen demand has been calculated. The carbonaceous oxygen demand was found to be 4,090 mg/liter and the nitrogenous oxygen demand 50,700 mg/liter. To obtain a completely stabilized product from urine, approximately 55,000 mg/liter of oxygen would be necessary. Experimental analyses during this research project have indicated an average chemical oxygen demand of 8,350 mg/liter and a nitrogenous oxygen demand of 42,500 mg/liter based on samples from five subjects.

Water from respiration and perspiration constitutes another source of human waste water. Although the major mineral constituent of this water is

TABLE I.- APPROXIMATE AVERAGE CONSTITUENTS OF URINE; AFTER MCKEE (REF. 4)

Determination	mg/day	mg/liter
Water	1,400,000	--
Total Solids	53,200	38,000 ^a
Inorganic		
Calcium	230	164
Magnesium	95	68 ^a
Sodium	4,200	3,000 ^a
Potassium	2,380	1,700
Bicarbonate	140	100
Chloride	7,000	5,000 ^a
Sulfur	1,120	800
Phosphorus	840	600
Trace elements		
Aluminum	0.077	0.055
Arsenic	0.023	0.016
Copper	0.035	0.025
Fluoride	1.54	1.10
Iron	0.49	0.35
Lead	0.028	0.02
Manganese	0.049	0.035
Nickel	0.21	0.15
Selenium	0.035	0.025
Silicon	9.10	6.5
Tin	0.013	0.009
Zinc	0.36	0.26
Organic		
Urea	30,000	21,400 ^a
Other N-compounds	4,700	3,360 ^a
Amino acids	2,100	1,500
Creatinine	1,600	1,140
Hippuric acid	350	250
Uric acid	140	100
Bilirubin	50	36
Creatine	56	40
Indican	70	50
Imidazole derivatives	170	121
Purine bases	42	30
Miscellaneous	140	100
Lipids, carbohydrates, and misc. organic acids	2,320	1,650
Carbonic acid	190	136
Citric acid	800	570
Formic acid	60	43
Lactic acid	210	150
Oxalic acid	35	25
Reducing substance	1,000	715
Miscellaneous	25	18
Vitamins, metabolites	50	36
Hormones	100	71

^aExcessive

sodium chloride, and small quantities of organic compounds arise from sweat and sebaceous gland excretions, the water that leaves the body and enters the atmosphere is the result of evaporative processes and, consequently, almost pure H_2O . It will appear as the condensate from the cooling system. The mineral and organic solids remain on the skin, to be dealt with later in wash water.

In this analysis, it is assumed that feces will be collected separately from urine and stored indefinitely.

A final major source of waste water consists of wash water for sponge baths, laundry, and cabin cleaning. The exact nature of cabin washings cannot be readily ascertained. It is assumed that such wastes will not exert a significant carbonaceous or nitrogenous oxygen demand. Bathing and laundry wastes will contain body secretions and probably a large amount of detergent. Based on data presented by Wallman et al. (ref. 7) and Ingram (ref. 2) the active detergent concentration of wash water is estimated to be about 700 mg/liter. Detergents typically contain 60 to 70 percent carbon. Assuming 60 percent carbon, the carbonaceous oxygen demand from this source alone is calculated to be 1120 mg/liter.

The probable amounts of minerals and organic materials removed daily from the skin of an average adult by washing have been listed by Breeze (ref. 6) and shown herein as table 11. Assuming that these quantities are contained in 5.5 liters of combined bathing and laundry water, the concentration of carbonaceous and nitrogenous compounds will be 161 and 75 mg/liter, respectively. From these compounds and their concentrations, it is estimated that the nitrogenous oxygen demand will be approximately 159 mg/liter and the carbonaceous oxygen demand will be approximately 285 mg/liter. Adding 1120 mg/liter from detergents, the total carbonaceous oxygen demand will be about 1405 mg/liter.

The estimated quantities and composition of the various waste waters can now be summarized in table III. Thus, the total oxygen demand is estimated to be 8620 mg/liter. This value can be compared with the oxygen demand of the settled sewage presently being fed the biological sand filters. Analysis of the influent feed indicates a chemical oxygen demand of 200 to 250 mg/liter. The total nitrogen, principally organic and ammonia nitrogen, in the settled sewage ranges from 30 to 45 mg/liter as nitrogen. Nitrogenous oxygen demand, therefore, amounts to 138 to 206 mg/liter. Using the maximum values, the total oxygen demand is 450 mg/liter. The waste waters occurring during space travel are seen to be about 19 times as strong as municipal waste waters with respect to the total oxygen demand. Recycling of a portion of the wastes can reduce this discrepancy by a factor of 2 to 3.

In addition to the problem of an extremely strong waste water, the relative composition of the waste must be examined. For terrestrial treatment operations the ideal ratio of carbon to nitrogen is often given as 100:16. This value reflects the average composition of living organic matter. In actual practice, the nitrogen component in sewage is often present in a relatively higher abundance. The settled sewage feed has a carbon-nitrogen

TABLE II. - PROBABLE AMOUNTS OF MATERIAL REMOVED DAILY FROM THE SKIN OF AN AVERAGE ADULT BY WASHING [AFTER BREEZE (REF. 6)]

Component	mg/day
Minerals	1911
Sodium	800
Potassium	300
Chlorine	800
Calcium	10
Magnesium	1
Carbonaceous compounds	886
Free fatty acids	420
Lactic acid	250
Cholesterol	75
Glucose	50
Squalene and paraffins	19
Triglycerides	50
Waxes	22
Nitrogenous compounds	411
Urea	350
Total amino acids	34
Creatinine	20
Uric acid	7

TABLE III. - ESTIMATED PER CAPITA OXYGEN DEMAND OF WASTE WATERS IN SPACE ENVIRONMENT

Source	Volume of waste, ml/day/man		Oxygen demand		
	Range	Value used	Nitrogenous, mg/liter	Carbonaceous, mg/liter	Total, g/day/man
Urine	600-2100(8)	1400	50,700	4090	76.8
Condensate from cool- ing system		1000(6)	0	0	0
Bathing	1500-4500(2)	2000	159	1405	3.1
Laundry	3000-4000(2)	3500	159	1405	5.5
Cabin cleaning	1000-5000(2)	2000	0(?)	0(?)	0(?)
Total		9900			85.4

Numbers in () are references.

ratio of about 2.5:1. From the data in table III, the carbon-nitrogen ratio of the waste water in space environments is calculated to be roughly 1:1.2. The effects of this ratio of constituents need to be investigated fully. Perhaps the inclusion of homogenized feces to the wastes will prove to be necessary to effect an optimum carbon-nitrogen ratio.

It should be noted that the problems associated with the high strength and the carbon-nitrogen ratio of the waste waters to be encountered in space travel are not unique to intermittent sand filtration. Rather, they are associated with all aerobic treatment processes and should be evaluated for each process. These problems increase in magnitude if one envisions the treatment of urine alone. The total oxygen demand rises to 55,000 mg/liter and the ratio of carbon to nitrogen becomes about 1:1.6. Although potable water may be recovered from wash water alone by physicochemical techniques, such water may better be used to dilute urine before aerobic stabilization and to provide carbonaceous compounds for more effective biodegradation.

With optimum operating conditions and proper acclimatization, it is anticipated that intermittent aerobic sand filters will yield a stabilized effluent when dosed with the waste waters encountered in space vehicles. Before such a system can be considered practical, additional research of the type being undertaken at the California Institute of Technology is needed.

RESEARCH OBJECTIVES

The laboratory studies presently being planned can be summarized in a few paragraphs. Since urine is the major load factor in space vehicle wastes, the principal experiments will be concerned with the treatment of this waste water. From the Whittier Narrows investigation (ref. 5), intermittent aerobic sand filters can degrade all synthetic detergents almost 100 percent. Addition of these compounds in the wash water is not expected to affect the treatment of urine adversely. On the contrary, beneficial effects might be observed owing to the improved carbon-nitrogen ratio.

Inevitably, the question arises as to whether to use natural urine or a synthetic urine for such research. Synthetic urine has certain advantages, particularly that of constant composition. Some investigators, however, have indicated that the artificial fluid does not behave like natural urine. To model exactly the composition of natural urine, it would be necessary to add well over 100 compounds to water. Using only the major constituents, synthetic urine differs significantly from natural urine in its physical and chemical behavior. The buffering capacity, for example, is greatly reduced. With these facts in mind, natural urine has been selected for this research project.

The initial studies with natural urine will begin very shortly in order to acclimate the intermittent sand filters to this feed. Several ripened columns will be dosed with full-strength urine. The remaining systems will be fed urine at dilutions ranging from 1:2 to 1:100. Once the biological system

become adapted to this new waste the effects of other parameters will be examined. In particular, the dose rate and airflow rate will be varied. In this manner, optimum operating conditions can be established.

Another series of studies to be made in the future concerns the breakdown of specific components of urine in the biological filters. Urea is the major organic constituent of urine with an average concentration of about 21,400 mg/liter (see table I). Of the remaining organic material, creatinine at a concentration of 1,140 mg/liter is the most prevalent. These two compounds will be fed separately to sand columns acclimated to urine. The effluent will be carefully analyzed to ascertain metabolic information on the microbial degradation of urea and creatinine.

While it is not possible to study the effects of zero gravity directly, the intermittent sand filters can be arranged to minimize the effects of gravity. If the columns are placed in a horizontal or nearly horizontal position, the influence of gravity on the transmission of the urine through the column can be almost entirely eliminated. The distribution and flow of the waste water through the column will be controlled primarily by capillary forces and the airflow.

In conjunction with this research project, Mr. Albert Pincince is conducting a detailed study of oxygen transfer in intermittent sand filters to elucidate the mechanisms of oxygen transport in such treatment systems. The diffusion of oxygen into the sand matrix and to the microorganisms is being evaluated.

As a final area of study, the effects of supplemental carbon on the stabilization of the waste waters will be ascertained. The implications of this work are apparent from the earlier discussion. Adding carbonaceous matter either from detergents or feces may be beneficial in the treatment of urine.

CONCLUDING REMARKS

As the manned space program begins to shift its emphasis to flights of long duration, many unique problems must be solved with respect to the space habitat. One of the most vexing of these problems is the supply of potable water for the space travelers. The most reasonable approach to water recovery systems would appear to involve an aerobic, biological process to stabilize the organic waste waters followed by some type of physical and/or chemical technique to produce the final, high-quality drinking water.

One aerobic treatment system that has not been investigated for space vehicles is intermittent filtration through fine porous media. This process offers certain inherent advantages in a zero-gravity environment. Although intermittent sand filtration is known to be an effective method of treating municipal waste waters, its ability to produce a stabilized effluent from the wastes to be encountered in space vehicles has not been evaluated. The

effects of dose rate, concentration of organic matter, and airflow rate must all be examined. In spite of the lack of experimental information, intermittent sand filtration does hold promise for use in space environments. The possibility that such a system can solve some of the problems of waste disposal in space is worthy of thorough investigation.

REFERENCES

1. Slonim, A. R.; Hallam, A. P.; Jensen, D. H.; and Kammermeyer, K.: Water Recovery from Physiological Sources for Space Applications. Final Rep. MRL-TDR-62-75, Wright Patterson Air Force Base, July 1962.
2. Ingram, W. T.: Microbiological Waste Treatment Processes in a Closed Ecology. AMRL-TDR-62-126, New York Univ., Jan. 1961-Mar. 1962.
3. Wallman, H.; and Barnett, S. M. : Water Recovery Systems (Multi-Variable). WADD TR 60-243, General Dynamics Corp., March 1960.
4. McKee, J. E.: Liquid Wastes and Water Potability in Space Vehicles. Conference on Nutrition in Space and Related Waste Problems, NASA SP-70, 1964.
5. McMichael, F. C.; and McKee, J. E.: Report of Research on Wastewater Reclamation at Whittier Narrows. California State Water Quality Control Board, Sacramento, 1965.
6. Breeze, R. K.: Space Vehicle Environmental Control Requirements Based on Equipment and Physiological Criteria. ASD TR 61-161, part 1, U. S. Air Force, December 1961.
7. Wallman, H.; Steele, J. A.; and Lubitz, J. A.: Multi-Filter System for Water Reclamation. Aerospace Med., vol. 36, Jan. 1965, pp. 35-39.
8. Altman, P. L.: Blood and Other Body Fluids. Ch. XIX, table 122, Committee on Biological Handbooks, Fed. Am. Soc. Exp. Biol., Washington, 1961.

THE SPIN-INERTIA CULTURE SYSTEM IN WASTE TREATMENT IN
CLOSED SYSTEMS

Clarence G. Golueke and William J. Oswald

Sanitary Engineering Research Laboratory
College of Engineering and School of Public Health
University of California, Berkeley

An essential characteristic of many of the biological systems intended for use in life support in closed environments is the aerobic breakdown of the wastes by bacteria and other microorganisms. As the statement implies, the efficacy and efficiency of such systems increase with increase in the number of microorganisms functioning at the maximum activity permitted by their genetic makeup and at the rate at which needed oxygen is made available to these organisms. Therefore, the objectives of research in biological systems generally are: (1) to determine the nature of those conditions under which the desired bacteria function most effectively, (2) to develop methods of establishing these conditions, (3) to find ways of maintaining dense cultures of the organisms under these conditions, and (4) to make oxygen available to the organisms in such a manner that it does not limit their activities. Dense cultures are needed because, in space applications, volume and weight must be kept at a minimum. Since the major factor with respect to weight of a bacterial culture is the water in which the organisms are suspended, this constituent is obviously the one to be reduced. Supplying oxygen in an available form is one of the major problems in biological stabilization of wastes, that is to attain a oxygen transfer rate from ambient gas to cell at a pace commensurate with the oxygen needs of the microorganism. Among the principal methods of accomplishing this transfer are: (1) bubbling air through the culture and (2) agitation. A third method of supplying oxygen is by photosynthesis, that is, photosynthetic oxygenation. Plants usually employed in this latter method are the planktonic algae.

The principal subject of this paper is a survey of the research done in our laboratory to attain the four objectives listed above.

Our approach to the problem of waste treatment in space took the form of attempting to miniaturize the system we had developed for terrestrial applications. This system essentially consists in growing a symbiotic culture of algae and bacteria and associated microorganisms in liquid or liquefied wastes. The role of the nonphotosynthetic members is to decompose the organic fraction of the wastes to forms that can serve as nutrients for the algae. The algae, in turn, convert these transformed wastes to algal cellular material and release oxygen for use by the nonphotosynthetic organisms. Algae are barvested and fed to livestock. Inasmuch as the unstable organic matter is converted to algalbiomass, which is removed from the system, the waste waters have, in effect, been converted to usable waters. An advantage of the system with regard to the waste treatment aspect per se is the manner in which the waste waters are oxygenated. Rather than depend upon the diffusion of

atmospheric oxygen, oxygen is obtained from the water itself and is produced in a molecular form distributed throughout the liquid since the photosynthesizing algae are dispersed throughout the culture medium.

Although the system works very well in terrestrial applications, certain difficulties stemming from the reliance upon photosynthesis for oxygen are encountered in applying it to space environments. The difficulties are too well known to warrant further belaboring, except to state that they arise from the need to illuminate the culture, and when a photosynthetic gas exchange system is used to bring CO_2 into the culture and to remove oxygen from it. To ensure most efficient use of light, shallow cultures are required -- about 3.5 mm deep. Unfortunately, the shallower the culture, the more surface area is required, so that even at very small culture volumes, the surface area may be quite large. Moreover, the use of shallow (film) cultures becomes a complicated operation when it comes to providing gas exchange. One can easily imagine the difficulty in forcing gas through a sheet of culture 3.5 mm deep (thickness) and encased in a conventional growth unit.

Rather than abandon as impractical the concept of using photosynthesis in waste treatment in life support systems, we sought to solve the attendant problems by developing a growth unit that would minimize or even eliminate them. Such a unit would have to depart radically from a conventional design. Our search led to the development of the algaatron system, namely, one in which a culture is grown as a film on the wall of a mechanically rotated drum. If the culture were algae, the drum would be transparent. In retrospect, our choice of the name "algaatron" was unfortunate in that it implies a limitation to algal culture, whereas the system is applicable to any situation in which instant mixing and a high rate of gas exchange are desired. Perhaps a better designation would have been the one used in the title of this paper, that is, "spin-inertia culture system."

Since detailed descriptions of the principles involved in the design and operation of the algaatron have been presented in previous papers (refs. 1 and 2), only a brief description is given at this time. As stated above, it consists of a mechanically rotated drum. As the drum rotates, the frictional drag of its wall on the culture imparts a rotation to the culture that is essentially equal to its own. Under the applied forces, the culture suspension flows out of a reservoir and spreads as a vertical sheet upon the inside of the rotating drum. The algaatron is operated as a chemostat by the installation of influent injecting and effluent decanting devices. The influent system can consist of any suitable injection mechanism. The effluent system preferably should be a decanting scoop positioned such that it is a distance from the drum wall equal to that of the depth of the culture when it has reached the desired volume. Any injection of media that increases the volume of the culture such that its depth exceeds the clearance between the scoop and wall results in the removal of the excess liquid. An extremely effective mixing is accomplished by placing a probe in the path of the rotating liquid. The introduction of the probe creates a wake where turbulent mixing occurs and thus brings about rapid and continuous renewal of the culture surface and consequent gas exchange between culture and ambient atmosphere.

The principles of the design and operation of a horizontally oriented algatron are much the same as those for a vertical system. The chief differences are those concerned with the vertical climb of the liquid and the reservoir design.

EXPERIMENTS

In our research during the past two years, we have tried to improve the design of the algatron system and investigate the efficacy of various models of the system with respect to algal yield, algal nutrient removal, waste treatment, and reaeration. Four different models have been developed and used, three vertical and one horizontal.

Materials and Methods

The first model of the algatron was a simplified version, with the drum 30 cm in diameter and 25.4 cm high. Since it was used solely for experiments concerned with algal yield, a full description of which has been published (refs. 1 and 2), no further mention of the unit will be made in this paper. The second model was constructed of Plexiglas according to the design diagrammed in figure 1. As indicated by the diagram, a trough is placed at the base of the vertical wall of the unit.

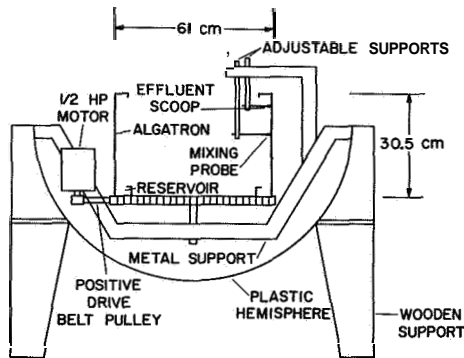


Figure 1. - Algatron, second model.

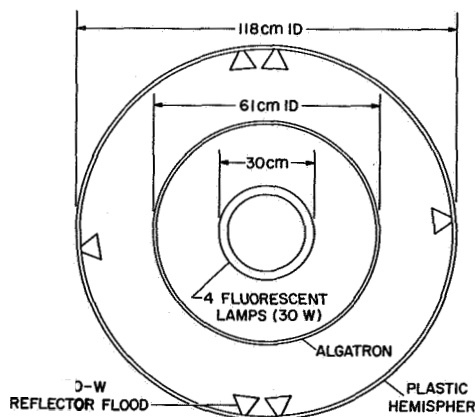


Figure 2. - Lamp arrangement around algatron, top view.

The trough serves as a reservoir for the algal suspension when the unit is not revolving. In earlier experiments, this algatron was completely enclosed by two plastic hemispheres designed to act as a light chamber. However, the chamber proved to be ineffective as a light diffuser, and in later experiments the upper hemisphere was removed and the lighting arrangement shown in figure 2 was substituted. The upper limit of the number and the wattage of the lamps in the second arrangement were determined by the rate of heat loss from the culture. With the number and intensity of the lamps shown in figure 2, the temperature of the culture varied from 32° to 35° C. The rotation of the drum, as well as the presence of a large specific liquid-air surface of about 1 cm²/cm³, served to prevent the buildup of heat that normally would have accompanied the pouring of an equivalent amount of light energy into a culture growing in a conventional unit, unless the latter were equipped with an elaborate cooling system.

The third version of the algatron system differed from the second mainly in the height of the drum, which was 1.23 m. The essentials of its design are shown in figure 3. Because of the height of the drum, provision had to be

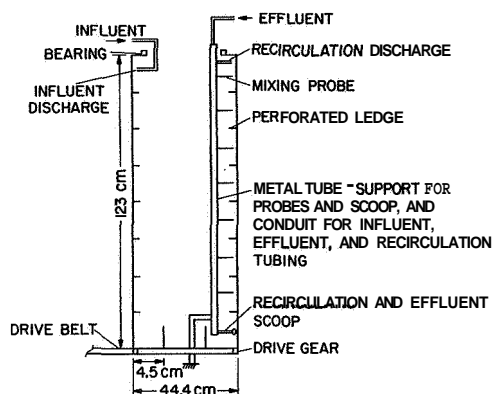


Figure 3.- Algatron, third model.

made for transporting culture from the base to the top of the unit, and the drum interior had to be equipped with perforated ledges so that the culture could be distributed uniformly from top to bottom. Distribution was accomplished by scooping up the culture at the base of the unit and discharging it just above the top ledge. Perforations in the ledges allowed the culture to return to the bottom of the unit. A measured portion of the recirculating liquid was discharged as effluent by inserting a "T" in the recirculation line and diverting a determined amount

of the liquid to the exterior. The energy required for moving the liquid through the recirculation and effluent lines was that imparted to the liquid by the rotation of the unit. The arrangement of recirculation scoop, mixing probes, influent and recirculation discharges are shown in figure 3. In the experiments described in this paper, two such units were operated in tandem. By force of necessity, the units were rather inadequately illuminated by five 500-W G. E. "Quartzline" lamps arranged in a semicircle in front of the two units. A major disadvantage with this light arrangement was that the culture could neither be bilaterally illuminated nor irradiated uniformly from top to bottom. For example, light intensity ranged from 45 ft-c at the bottom and top extremities to 3000 ft-c over a 15-cm band at a zone midway up the cylinders and directly in line with the lamps.

The fourth model, a horizontal drum 15 cm in diameter and 13.5 cm long, was used to determine the reaeration rate of the liquid contained in the unit. The rates obtained may be used as indicators of the extent of gas exchange that can be expected in such a unit.

'Domestic primary sewage was used as the suspending medium. In some experiments, the sewage was enriched and blended (Waring blender) with fresh feces and urine in amounts called for by the experiments; in others, with urea (300 mg/liter). Except in experiments with the first model, in which a 1 percent CO₂ concentration was kept in the gas to bathe the culture, ambient air and the respiratory gas of bacteria constituted the CO₂ sources.

The predominant algal species generally belonged to the Chlorella group, although no attempt was made to control algal or bacterial types. Under certain circumstances, that is, those in which retention time of the biomass was prolonged, Oscillatoria predominated. No attempt was made to identify the bacteria. However, because of the nature of the medium, it is safe to assume that they were those normally associated with human wastes.

RESULTS

Second Model of the Alгатron, Yield

The principal factors studied with the second model were liquid and biomass detention periods and light intensity. The results obtained in the experiments are listed in table I. The general uniformity of light intensity

TABLE I. - ALGAL YIELD IN THE SECOND MODEL OF THE ALGATRON

Run ^a	Light intensity ^b (ft-c)				Detention period (days)		Yield	
	Outside surface'		Inside surface ^d					
	Min	Max	Min	Max	Liquid	Biomass	g/liter/day	g/m ² /day ^e
1	150	150	165	165	1.6	1.6	0.31	3.24
2	150	150	165	165	1.0	1.0	.36	3.82
3	150	150	165	165	.75	.75	.42	4.45
4	200	200	230	230	1.8	1.8	.74	7.81
5	200	200	230	230	1.0	1.0	.99	10.54
6	200	200	230	230	.75	.75	1.26	13.33
7	225	225	270	270	1.0	1.2	1.29	13.62
8	225	225	270	270	.75	.95	1.50	15.87
9	100	6000	800	800	.44	3.0	1.66	14.77
10	100	6000	800	800	.44	2.0	1.71	15.22
11	100	6000	800	800	.36	.75	2.74	23.45

^aSee text for important environmental conditions other than those listed in the table.

^bAt culture surface.

^cSurface of culture facing lamps external to drum.

^dSurface of culture facing fluorescent lamps inside.

^eBased on only one of the two illuminated surfaces of the culture.

in the first eight experiments was due to the fact that the alгатron was enclosed in a light chamber. The ineffectiveness of the chamber is indicated by the low light intensities at the culture surface despite the use of five 500-W "Quartzline" lamps as the light source. The minimum light intensities listed for the last three experiments (upper hemisphere removed) were those prevailing between the incandescent lamps, and the **maximum** were those in a zone (about 15 cm in diameter) directly in front of the lamps.

The removal of the upper hemisphere, as well as the unusually complete mixing of the culture, resulted in a very high rate of evaporation. For example, from 75 to 95 percent of the rapid turnover of liquid, indicated by the short detention periods in experiments 9, 10, and 11, resulted from the removal of water from the culture by evaporation.

As the data in table I indicate, daily yield of algae per liter of culture increased with increase in light intensity and also with decrease in detention period of the biomass. The lower ratio between yield per square meter of surface and yield per liter in the first eight runs was due to the fact that the volume of the culture was 5.2 liters in runs 9 and 10 and 5 liters in run 11, as compared to 6.2 liters in the first eight runs.

Second Model, Organic and Nutrient Reduction

The extent of the organic and nutrient removal from the influent wastes with the second model is indicated by the data in table II. The influent

TABLE II.- ORGANIC AND ALGAE NUTRIENT REDUCTION WITH THE SECOND MODEL

Run	Human proportion (organic)	BOD - 5-day		
		Influent (mg/liter)	Effluent (mg/liter)	Reduction (percent)
1	0.185	242	14	94
2	.222	288	18	94
3	.444	530	65	88
4	.666	810	79	90
Run	Human proportion (organic)	Kjeldahl-N		
		Influent (mg/liter)	Effluent (mg/liter)	Reduction (percent)
1	0.185	146	55	62
2	.222	170	30	82
3	.444	310	62	80
4	.666	450	152	66
Run	Human proportion (organic)	PO ₄		
		Influent (mg/liter)	Effluent (mg/liter)	Reduction (percent)
1	0.185	58	31	47
2	.222	66	43	35
3	.444	110	65	41
4	.666	146	69	53

medium used in this series of experiments consisted of sewage enriched with fresh human feces and urine. The amount of added wastes ranged from the equivalent of 0.185 to 0.666 of the daily output of one of the 75-kg subjects.

In analyzing the data on effluent quality and percent reduction, it should be remembered that the data are based on a material that became highly concentrated because of the loss of the greater portion of the incoming water (about 57 percent). The concentration of the various constituents in the absence of evaporation and with all of the water discharged as effluent can be estimated by multiplying the values given in table II for effluent concentration by 0.43. If such an estimate is used, the values for percent reduction in concentrations of the various influent elements will be correspondingly higher than those listed in the table, and from 0.038 to 0.155 m² of drum wall area will be required per kilogram of body weight to treat body wastes to the extent generally demanded in terrestrial applications. Although the biochemical oxygen demand (BOD) (5-day) obtained in experiment 10 was quite high (on the order of 23.4 g/m² of reactor surface), the BOD of the effluent was too high for direct use. This difficulty could be met by passing the effluent through a second algastron.

The calcium and magnesium concentrations of the influent and effluent were also determined. In terms of concentration as discharged from the algastron, the calcium concentration of the effluent was from 0 to 15 percent greater than that of the influent. However, if the evaporative losses are considered, a reduction of 36 to 55 percent occurs. The discharged effluent concentration of magnesium was from 13 to 18 percent greater than that of the influent. When evaporative losses are considered, the increase changes to decrease, and the reduction of incoming magnesium would range from 23 to 43 percent. Percentage reduction in calcium and magnesium increased with increase in influent concentration of these elements.

Second Model, Water Regeneration

Water evaporation rates were measured regularly. This evaporated water may be condensed and used to meet the water requirements of the crew of a space vehicle; inasmuch as it is a low temperature distilled water, it is free from the objectionable distillates that would be present in water produced by conventional high temperature distillation of untreated wastes. During a three-month period of observation, the rate was 1.6 liters/liter of culture volume, or 17.3 liters/m² of culture surface. The high rate of regeneration obtained in the experiments indicates that this function can be easily carried on in an integrated system.

Third Model, Algae Nutrient Removal

Experiments completed with the third model of the algastron were concerned mainly with nutrient removal since inadequate lighting made studies concerned with yield impractical. As stated before, two units of this model were built and operated in tandem. Unit B was equipped with a blower for forcing air through the interior of the column. Because of the absence of an interior ventilating system, the rate of evaporation from unit A was much less than that from unit B.

Data obtained during the experiments are listed in table 111. The volatile dissolved solids of the incoming medium were reduced by 29 percent

TABLE 111.- ALGAE NUTRIENT REMOVAL IN THE THIRD MODEL

Item	Influent ^a (mg/liter)	Final effluent ^a (mg/liter)		Reduction of material ^a (percent)	
		unit A	Unit B ^b	Unit A	Unit B ^b
Total dissolved solids	374	394	320	0	15
Volatile dissolved solids	150	107	85	29	43
Total nitrogen	107	31	28	71	74
NH ₄ -N	51	8	9	84	82
Organic -N	56	23	9	59	84
Calcium	29	2	17	80	42
Magnesium	33	31	39	7	0
PO ₄	15	7	7	53	53

^aAverage values of four experimental runs.

^bUnit B was equipped with a blower for forcing air through the length of the drum.

in unit A and 43 percent in B; total nitrogen by 71 to 74 percent; NH₄-N by 84 to 82 percent; Ca by 80 percent in A and 42 percent in B; and PO₄ by 53 percent in both. Magnesium concentration was decreased 10 percent in unit A and increased 18 percent in unit B.

Fourth Model, Reaeration

Since one of the advantages of the alga-tron is the high rate of gas exchange possible, a study was made of the rate of aeration that could be accomplished in the unit. An alga-tron with a horizontally oriented drum was used. Except for feed and gas inlet and effluent ports, the drum was sealed at both ends. Except for the use of 24-hour settled sewage in one series of experiments, boiled distilled water was used as the liquid in most of the experiments. The sewage was used because its oxygen demand would increase the amount of oxygen required to reach saturation and thus provide a wider range of oxygen uptake than was possible with distilled water. Oxygen uptake rates were determined with the drum stationary and with it rotating, the former serving as a control. Liquid was passed through the system at rates equivalent to detention periods of 0.66 to 5.2 minutes. (The shortest possible detention period with the equipment was 0.66 minute.) Two volumes of liquid were used, 500 and 750 ml. The surface-to-volume ratio of the stationary liquid at the former volume was 0.36 and at the latter, 0.26.

The results obtained in the experiments are listed in table IV. As the data indicate, with the distilled water volume at 500 ml (21 percent of the

TABU IV.- RATE OF OXYGENATION IN THE HORIZONTAL ALGATRON

Run	Influent liquid," Dissolved oxygen		Detention period (min)	Volume of liquid in unit (ml)	Surface- to- volume ratio	Stationary		Rotating	
	(mg/liter)	(percent saturation)				Dissolved oxygen		Dissolved oxygen	
						(mg/liter)	(percent saturation)	(mg/liter)	(percent saturation)
1	2.9	27	0.75	750	0.26	4.1	42	8.4	87
2	2.9	27	1.2	750	.26	4.5	45	9.3	100
3	2.3	22	.66	500	.36	5.0	56	9.6	101
4	2.3	22	1.0	500	.36	4.7	48	10.6	105
5	2.3	22	2.1	500	.36	4.6	48	10.6	106
6	.6	5	.66	500	.36	1.6	17	7.7	83
7	.6	5	1.0	500	.36	2.5	26	9.3	98
8	.6	5	3.6	500	.36	2.6	26	9.5	98

volume of the drum), rotating the drum brought about a dissolved oxygen concentration slightly in excess of saturation at all detention periods tried. Saturation ranged from 48 to 56 percent with the drum stationary. At a volume of 750 ml (31 percent of drum capacity), 100-percent saturation was reached at the 1.2-minute detention period when the drum was rotating, and 45 percent when stationary. Lengthening the period to 5.2 minutes increased saturation to 49 percent when the drum was stationary, and 104 percent with the drum rotating. The rate of reaeration was considerably less when sewage was used and the drum kept stationary, ranging from 17 to 26 percent at detention periods of 0.66 to 3.6 minutes, and from 83 to 98 percent with the drum rotating. The volume of the liquid was 500 ml in both cases.

DESIGN OF AN INTEGRATED SYSTEM

Using the results obtained in the experiments, especially those listed in table II, it is possible to design an integrated biological system for a "standard" astronaut. The design, summarized schematically in figure 4, considers waste treatment, nutrient removal, and water production. It is not complete in that it does not include oxygen production (for human consumption) and CO₂ absorption. According to the design, the daily wastes of a single astronaut would be converted to 21 liters of liquid wastes having a 17-g BOD (>-day), 9.5 g of nitrogen, and 3.1 of phosphate. This liquid would be applied to a primary algatron having a culture volume of 7.5 liters or 0.7 m² wet (culture) surface area. Effluent from the primary drum, approximately 0.1 liter in volume (less in volume than that of the influent because of evaporation), would be applied to a secondary algatron. The drum area of the

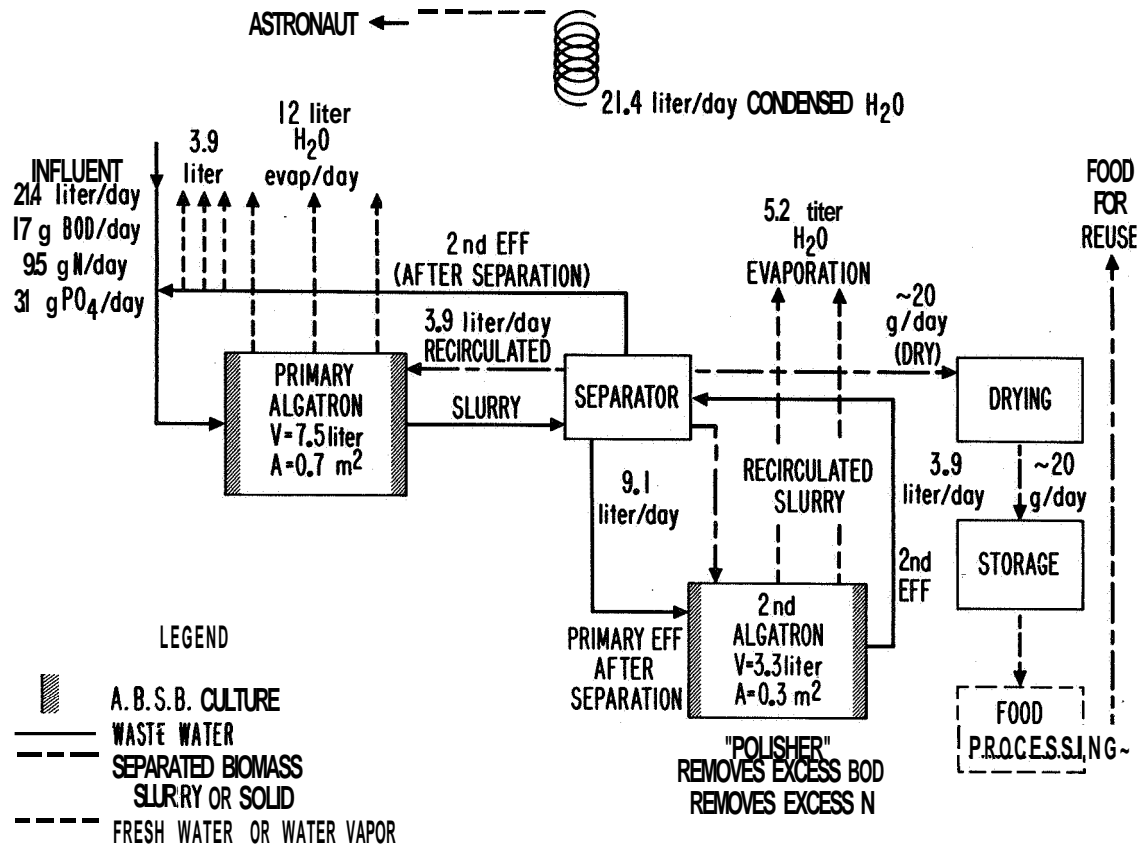


Figure 4.- Schematic diagram of integrated system.

secondary algalton would be 0.3 m². Thus, the total drum area would be 1 m² and the total culture volume, 10.3 liters.

Effluent from the secondary algalton, about 3.9 liters in volume, would be mixed with the wastes applied to the primary unit or evaporated to produce water and dry solids. The latter could be stored or subjected to further specialized decomposition. From 17 to 21 liters of low temperature distilled water would be produced each day by the system. The concentration of biomass would be maintained at 2000 mg/liter, preferably with a continuous recirculation of biomass operating in conjunction with an optical density sensing and control device.

A bulk of about 20 g of biomass would be removed from the system each day, dried, and then stored. In this way, carbon and other nutrients would be removed. Storage as well as treatment would be entirely odorless functions in the system as designed.

DISCUSSION

Problems

As is to be expected when dealing with any novel system, several problems of design and operation must be solved before the full potential of the alga-tron can be realized. One of the questions to be answered is that of the relative merits of the vertical over the horizontal drum, or vice versa. A vertical drum maybe easier to illuminate bilaterally. The culture would be more uniformly distributed along the length of a horizontal drum than up and down the wall of a vertical drum. However, separation of solid and liquid phases (i.e., maintenance of heavy concentration of biomass) might be difficult to maintain in a horizontal drum because of the washing action brought about by the interaction of the rotating wall and the pool of liquid that normally would be at the bottom of the drum. Construction design questions to be answered are the relative merits of large versus **small** units, and of tall versus squat units. Operational features to be more fully explored are those concerned with biomass concentration, detention periods, and internal recirculation.

Potential

We have only begun to explore the potential of the alga-tron with regard to waste treatment. The work thus far has been accompanied by very encouraging results. Thus, the experiments involving the treatment of human wastes indicated that the wastes of an individual could be sufficiently treated with the use of 3 or 4 m² of alga-tron surface area. As our experimentation progresses, these ratios of surface area to stabilized waste should decrease, since there are undoubtedly better operational procedures which we have not yet tried. One such procedure would involve the maintenance of a highly concentrated symbiotic (algae and bacteria) biomass under conditions permitting the application of very heavy organic and hydraulic loadings at very short detention periods with a minimum volume and weight of liquid within the reactor. If successful, such an arrangement would sharply reduce construction and power requirements.

A combination of the alga-tron system with a very dense, highly active symbiotic biomass should result in a treatment performance superior to that achieved in the light exposed part of a recirculating, trickling filter, since, in the alga-tron system, the extent of contact between wastes and biomass and the development of concentration of active biomass are greater, while the rate of oxygenation is higher than that in the symbiotic mass. The alga-tron system-active symbiotic biomass combination also offers the advantages of a high rate activated sludge system without most of its disadvantages. An intensity of oxygenation greater than that in an activated sludge system is attained because of a constant supply of oxygen from photosynthesis in the biomass, and from the surface aeration that is applied to the recirculated, instantly mixed, highly concentrated biomass. Moreover, the degree of control of sludge separation for recirculation and disposal is greater than that in the activated sludge system, a higher biomass concentration is attained in the reactor, and a higher mass yield is possible.

Because of its design and operational characteristics, the alga-tron may prove useful in the breakdown of the residues that constitute a difficult problem in the application of biological systems to waste treatment in closed systems. The application could be made by concentrating the residues and passing them through an alga-tron containing a culture of the organisms capable of utilizing the residues as a substrate. Constituents of these residues are the more complex polysaccharides, lignins, chitin, and certain pigments. Lignins and chitins are readily decomposed by certain fungi, some of which are filamentous, and most of which are aerobic in metabolism. The alga-tron is admirably suited to the culture of filamentous organisms, since the filaments are held against the wall of the unit by centrifugal force, and yet are continually bathed by the waste waters receiving treatment.

CONCLUSIONS

A consideration of the intrinsic features of the alga-tron and of our experience with it as described in this paper furnish sound reasons for believing that the alga-tron can be used to advantage in waste treatment in closed systems. A list of the principal characteristics of the alga-tron system serves as our closing statement: With the alga-tron system: (1) a thin-layer culture can be maintained with a minimum of hardware and supporting equipment; (2) in cultures of photosynthetic organisms, a high illuminated surface-to-volume ratio is provided; (3) a very high rate of gas exchange is accomplished with a minimum expenditure of energy because of the high surface-to-volume ratio, of the vigorous and instant mixing, and surface renewal; and (4) a heavily concentrated biomass can be maintained under optimum conditions with very little expenditure of hardware, supporting equipment, and energy.

ACKNOWLEDGEMENTS

The research reported herein was sponsored in part by the Air Force Cambridge Research Laboratories, Office of Aerospace Research, under Contract No. AF 19(628)-2462, and by the Sanitary Engineering Research Laboratory of the University of California.

REFERENCES

1. Golueke, C. G.; Oswald, W. J.; and Gee, H. K.: A Study of Fundamental Factors Pertinent to Microbiological Waste Conversion in Control of Isolated Environments. First Tech. Rep. SERL-64-5, San. Eng. Res. Lab., Univ. of Calif., Berkeley, 1964.
2. Golueke, C. G.; and Oswald, W. J.: The Alga-tron - A Novel Microbial Culture System. Sun at Work, vol. 11, 1966, pp. 3-9.

DESIGN AND EVALUATION OF CHEMICALLY SYNTHESIZED
FOOD FOR LONG SPACE MISSIONS

By Jacob Shapira

Ames Research Center

A major consideration in terms of weight and volume for long space missions is the food requirements of the astronauts. Any potential method for regenerating a portion of the diet from metabolic products warrants investigation. Although considerable effort has been and is being made to evaluate bioregenerative systems for food production, the theoretically greater advantages of chemical synthesis of dietary components have attracted relatively little attention. This report will survey the literature on synthetic methods which might be adapted for the production of edible carbohydrate and fat in the space environment.

The selection of carbohydrate and fat for study was dictated by the fact that they serve as the major source of calories in the normal diet. Of the two, fat has a much higher caloric density and therefore smaller amounts would be required. It is possible that less than 10 percent of the total food requirements of the astronaut would have to be carried along and would supply only those components of the diet that are difficult to synthesize, such as essential amino acids, unsaturated fatty acids, and vitamins. Even these might be provided by incorporating into the diet relatively small amounts of material from a bioregenerative system.

CARBOHYDRATE

General Considerations

The carbohydrate in the normal diet usually provides more than 50 percent of the caloric requirements and consists mostly of polymerized D-glucose (starch, glycogen) with smaller amounts of free sugars and disaccharides. The major metabolizable monosaccharides are D-glucose, D-galactose, D-mannose, and D-fructose. All these sugars are catabolized to the common intermediate trioses D-glyceraldehyde and dihydroxyacetone and then to carbon dioxide and water. Most of the energy derived from carbohydrate is produced by this oxidation of trioses.

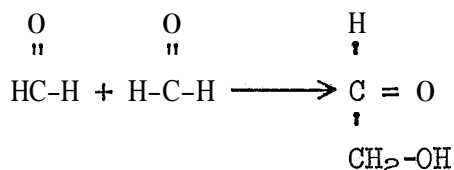
Although glycerol occurs primarily in fat, it can be considered a carbohydrate material since it is catabolized via the triose sugars. In fact, reduction of either D-glyceraldehyde or dihydroxyacetone gives glycerol.

Except for D-glucose, the content in the body of the various intermediates in the catabolism of carbohydrate is quite low. There is good evidence that, at least for a few of these intermediates, ingestion of substantial amounts is well tolerated and that they act physiologically and biochemically in much the same manner as carbohydrate.

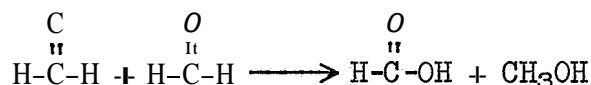
Simple chemical methods for the synthesis of carbohydrate are limited at present to the production of mixtures of sugars, although schemes have been proposed for the synthesis of individual compounds. A common chemical intermediate in the synthesis from CO₂ and H₂O is formaldehyde. For the sake of brevity, methods for preparing formaldehyde will not be discussed aside from the comment that several methods for its production in the space environment have been studied and the process appears feasible.

Condensation of Formaldehyde

As early as 1861, it was observed (ref. 1) that treating aqueous solutions of formaldehyde with dilute base gave rise to a complex mixture of materials whose major component was subsequently shown to be (ref. 2) an optically inactive mixture of sugars (formose). The nature of the individual components of the mixture was investigated intensively by Fischer and Passmore (ref. 3). Through the years, the chemical mechanisms by which the reaction occurs have been studied extensively. Apparently, the first product is glycolic aldehyde (ref. 4), which arises through a variation of the Aldol condensation and whose formation is probably the rate limiting step for the total reaction sequence:



A major competing reaction that must be minimized is the Cannizzaro reaction which leads to the production of formic acid and methanol:



However, since methanol seems to have a catalytic effect on the reaction (ref. 5), it is possible that a small amount of disproportionation is needed for induction. The relative rates of these two initial reactions depend greatly on the alkalinity of the reaction mixture and influence the choice of catalyst.

Once the initial two-carbon compound is produced, a rapid exothermic series of Aldol condensation reactions leading to formose takes place. The three carbon intermediates are primarily glyceraldehyde and dihydroxyacetone (ref. 6). Several schemes have been proposed for the further reactions. For example, Katzschmann (ref. 7) has proposed that a branched-chain reactive

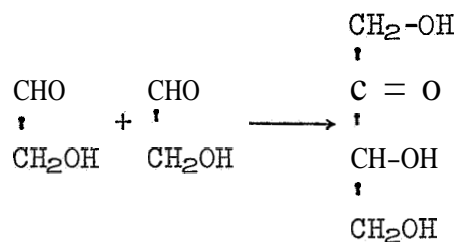
dimer of dihydroxyacetone is an obligatory intermediate. Others have proposed more complex schemes (ref. 8). In any case, the nature of the products is highly dependent upon the exact conditions and catalyst. This catalyst can be a variety of types, but in general must be capable both of providing slightly alkaline conditions in the medium and of complexing effectively with aldehydes. In a rather typical case, $\text{Ca}(\text{OH})_2$, at a molar ratio of 1:10 with 4 percent formaldehyde, gave the products shown in table I (ref. 5). Various accelerators, such as aldehydes and hexoses, seem to speed the reaction without altering the distribution of products. Conversely, the presence of some materials, such as Al_2O_3 or copper salts, has been reported (ref. 5) to abolish the reaction.

TABLE I.- COMPOSITION OF FORMOSE MIXTURE REPORTED BY AKERLOF AND MITCHELL (5)

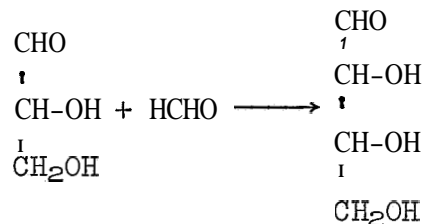
Percentage by weight of mixture	Possible identity
0.4	Glycolic aldehyde
1.1	Glyceraldehyde
1.8	Dihydroxyacetone
3.2	Erythrose, threose erythulose
2.5	Xylulose
6.5	Ribose, dendroketose
17.2	Xylose
16.5	Fructose, mannose
17.5	Sorbose, arabinose
16.8	Glucose
8.5	Galactose
4.4	Unidentified sugar
< 2	A heptulose

Trioses and Tetroses

When calcium carbonate and 2-percent aqueous formaldehyde are heated to 100° , a considerable amount of dihydroxyacetone and glycolic aldehyde is formed (refs. 4 and 9). Similar products are produced when ZnCO_3 is used (ref. 10). When sodium sulfite (which forms an addition compound with aldehydes) is used, a product is formed which is largely glyceraldehyde and dihydroxyacetone (ref. 11). Ketotetroses can arise from the Aldol condensation between two molecules of glycolic aldehyde, but the product is so reactive that its unique formation has not been reported.



An alternative condensation of a triose and formaldehyde would give the aldotetrose:



Pentoses and Hexoses

Reports of specific syntheses of pentoses and hexoses are sparse. Leow's original observation (ref. 2) was that a considerable proportion of the product was "hexose," but he did not adequately characterize the product. (He reported an empirical formula of $\text{C}_6\text{H}_{10}\text{O}_5$.) Arabinoketose can arise as the main product after prolonged heating of 2 percent HCHO with CaCO_3 (refs. 4 and 9). When alkaline PbO and MgSO_4 or $\text{Ca}_3(\text{PO}_4)_2$ were used, fructose, sorbose, and arabinoketose could be found. The most adequate study to characterize the product is that of Akerlof and Mitchell (ref. 5) where, as can be seen from table I, the majority of the product was a mixture of xylose, fructose, mannose, sorbose, arabinose, glucose, and galactose. It seems unlikely that the reaction can be made specific for any of these sugars, but it is conceivable that preferred hexoses might be isolated from the reaction product.

There are reports (refs. 12 and 13) that radiation of a variety of types can induce the condensation of formaldehyde. The nature of the product when ionizing radiation or coronal discharge is used is not clear. However, since these reactions occur in the gaseous phase, they offer significant practical advantages over liquid-phase reactions. Photoactivation of formaldehyde in the liquid phase using red light gives sugars. However, since this still requires a $\text{Ca}(\text{OH})_2$ catalyst with all the attendant difficulties of product separation, this approach does not appear attractive.

Continuous Synthesis of Formose Mixtures

An apparatus (fig. 1) was constructed here which permitted the continuous production of formose sugar. In the initial studies, the apparatus produced about 10 g of sugar per hour, the rate of production being limited by the initial reaction conditions. It was immediately observed that a fresh mixture of $\text{Ca}(\text{OH})_2$ with formaldehyde (1:10 molar ratio) would react relatively rapidly and exothermically at 60° to 65° , but that if the mixture was stirred at room

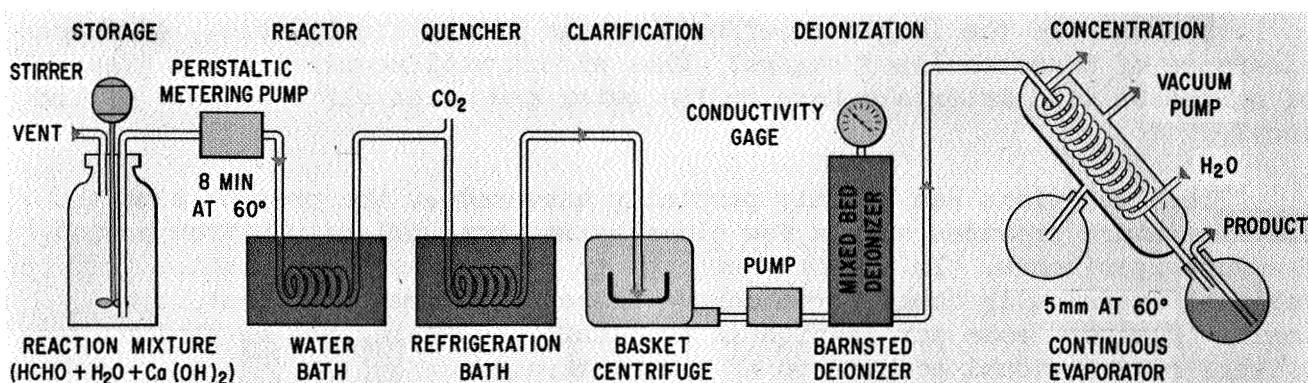


Figure 1.- Representation of laboratory unit.

temperature for 2 to 3 days, heating at 80° to 90° did not cause a reaction. As described in the literature, the addition of small amounts to the reaction product did enhance the reaction rate. Our most surprising, and as yet unexplained, observation was that adding small amounts of CaO to the reaction mixtures, whether they be fresh or quite old, enhanced the reaction rate tremendously. Using this technique, it was possible to generate formose sugar at the rate of about 100 g/hr, a tenfold increase over previously reported production rates. This may have been due, in part, to the use of a somewhat higher concentration of formaldehyde than has been used previously. At present, sufficient material is available to conduct extensive animal feeding studies.

It has been reported that the "formose" mixture is toxic to animals, presumably because of chemically bound formaldehyde liberated by the acid in the stomach. Although it is not certain that our product is toxic, efforts have begun to remove any formaldehyde that might be present. This has involved a mild acid hydrolysis of formose followed by high vacuum evaporation at 50° of all volatiles present. This purification seems particularly necessary since examination of the original mixture for glucose using the glucose oxidase technique indicates a concentration of less than 0.5 percent free D-glucose, whereas the literature (ref. 5) indicates the presence of 8 percent D-glucose as determined by paper chromatography. The best explanation available is that virtually all of the glucose is present as a formaldehyde hemiacetal derivative.

Metabolic Utilization of Formaldehyde Condensates

The wide spectrum of products produced by the condensation of formaldehyde raises some important questions as to the suitability of this material for nutrition. These include the following considerations:

- (a) A mixture of optical isomers is produced. Are the nonbiological L-forms metabolized; if so, to what extent? Are some of the more unusual sugars toxic or can they act as metabolic inhibitors?

(b) What are the long-term effects on the liver, kidney, etc., of forced clearance of nonmetabolized sugars? What effect **will** required renal clearance of nonmetabolized materials have on the water and electrolyte balance of the body?

These questions can be only partially answered at the present time and the literature provides only a few clues as to what will result from actual feeding experiments. In the only attempt to provide such information (ref. 5), residual organically bound formaldehyde caused the formose product to be toxic. However, some information is available concerning the metabolic effects of individual components of the mixture.

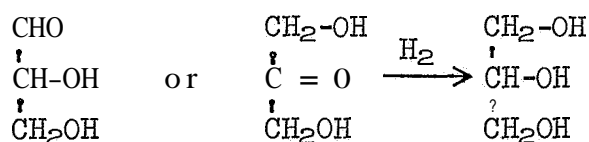
Metabolism of L-sugars. - In 1890, Fischer (ref. 14) reported that L-glucose was not fermented by brewer's yeast - an organism which has quite specific carbohydrate requirements. Subsequently, it was shown (ref. 15) that neither E. coli, B. aerogenes, nor surviving slices of rat brain and Sarcoma 39 could oxidize L-glucose and that there was no interference with the use of D-glucose. When L-glucose was injected into rats at a level of 1 g/kg, approximately 85 percent was excreted in the urine in 24 hours with no detrimental effects observed. The only further observations concerning L-glucose in the whole animal are that L-glucose has a slight salty taste and that L-mannose is less sweet than D-mannose (ref. 16). In plants, L-glucose is transformed into L-gluconic acid which accumulates. Administration of a variety of other D- and L-sugars did not result in the accumulation of the corresponding aldonic acid (ref. 17).

There are relatively few references concerning the metabolism of other L-monosaccharides, and apparently only one where the intact animal was used. Yamaguchi (ref. 18) has shown that isolated intestinal segments absorb both antipodes of glucose, galactose, mannose, and arabinose at about the same rate and with apparently the ~~same~~ degree of formation of sugar phosphate. However, there was no evidence of the formation of liver glycogen from any of the L-sugars (ref. 18) nor from either isomer of arabinose. These results are consistent with earlier reports concerning arabinose metabolism where glycogen formation in animals from either D- or L-arabinose was minimal (refs. 19 and 20). However, L-arabinose is fermented readily by a variety of bacteria, whereas the D-isomer is not (ref. 21). Isolated rat diaphragm can utilize D-glucose, D-mannose, and D-fructose for energy and can incorporate carbon from these into nucleic acids, whereas D-galactose, D-xylose, D-ribose, D- and L-arabinose, and L-sorbose are not utilized (ref. 22). However, rabbit leucocytes can convert D- and L-arabinose and D-lyxose to glycogen and lactic acid, presumably via fructose-6-P₀₄ (ref. 23).

Cellular transport of L-hexoses and pentoses.- The subject of mechanisms for the passage of sugars across all membranes has been of considerable interest for many years. Associated with these studies are observations concerning a wide variety of unusual sugars. The results of Yamaguchi (ref. 18) are difficult to reconcile with those of Rummel and Stupp (ref. 24) who report that at a mucosal concentration of 2.8 mM/L of D-glucose, 12 percent was absorbed in vitro in 2 hours whereas only 0.6 percent L-glucose was absorbed. This latter rate would be expected from passive diffusion. The water absorption from L-glucose solutions was 50 percent less than that from D-glucose

solutions. Other studies have been conducted on entrance of sugars into muscle tissue. In the isolated perfused rat heart, L-glucose does not enter the tissue even in the presence of insulin or anoxia (ref. 25), situations where the entrance of D-glucose is facilitated. Others have shown (ref. 26) that a competition for transport by isolated rat diaphragm exists between D-glucose, D-mannose, D-xylose, D- and L-arabinose, and D-lyxose, but not with a group of sugars which includes D-galactose, D-fructose, maltose, or D-sorbitol. More recently, it was shown (ref. 27) in isolated perfuse rabbit heart that penetration by L-arabinose is insulin sensitive and antagonized by phlorrhizin, suggesting an active transport mechanism.

Metabolism at trioses and glycerol.- As indicated previously, the condensation of formaldehyde can result in trioses, which might be fed as such or reduced with hydrogen to glycerol.



The possibility that compounds of this type are useful nutrients in animals devolves primarily from studies of the intermediary metabolism of carbohydrate where these compounds appear as active intermediates. Thus, Cori and Cori in 1928 (ref. 28) demonstrated that dihydroxyacetone administered orally is converted by the intact animal to glucose and glycogen, although an appreciable amount also appears in the urine. About 50 percent of the dose was oxidized, 20 percent deposited as glycogen, 15 percent excreted, and the remainder was unaccounted for. The degree of oxidation was greater than when glucose was given, although glycogen formation was somewhat less. These data are corroborated by the studies of Tsao et al. (refs. 29 and 30), who more recently administered radioactive dihydroxyacetone. They found that in 2 hours, 28 percent of the radioactivity from dihydroxyacetone appeared in the expired air as opposed to 47 percent from radioactive glucose. The acute and chronic toxicity of dihydroxyacetone is not available from the literature.

Information concerning glyceraldehyde is more limited. Preliminary evidence that it or glycerol could be converted to glycogen by the liver was reported by Cori and Shine (ref. 31). Later, Stohr (ref. 32) showed that feeding of 0.2 to 0.3 g of D, L-glyceraldehyde per 100 g body weight to fasting rats caused a rise in liver glycogen from 0.05 percent to 0.80 percent while blood sugar increased. Glyceraldehyde could no longer be detected in the blood 3 hours after feeding. However, there was no increase in muscle glycogen, whereas feeding dihydroxyacetone did cause an increase in this tissue (ref. 33). At a level of 0.2 mg triose per 100 g body weight, the absorption of D, L-glyceraldehyde in 3 hours was 54 percent, dihydroxyacetone was 68 percent, and pyruvic acid, 37 percent. The increase in liver glycogen was 63 percent for dihydroxyacetone, 50 percent for DL-glyceraldehyde, and 34 percent for pyruvic acid. These data are highly encouraging for the potential use of trioses as food. No problem occurred with the use of the DL-isomers of glyceraldehyde and presumably they are both utilized via dihydroxyacetone.

Glycerol offers extremely attractive possibilities as food. Man normally consumes 10 to 40 g/day of it as a component of dietary lipids. Humans have been given 100 g/day for 30 days with no ill effect. This represents approximately 20 percent of total caloric requirements derived from this material. As much as 300 g of glycerol has been given mixed with food, again without harmful effect. Rats can be maintained for months on a diet which provides up to 40 percent of the calories as glycerol without obvious effect on growth or health (ref, 34). Studies in progress here indicate that glycerol can serve as the sole source of carbohydrate in the diet of weanling rats.

FATS

General Considerations

Fats usually comprise 10 to 40 percent of the normal diet and are primarily mixtures of long-chain saturated and unsaturated fatty acid triglycerides. Catabolism of the fatty acids gives rise to acetate which is, in turn, oxidized. Whereas it is possible to increase the carbohydrate content of the diet to very high levels without deleterious effects, diets containing over 50 percent fat lead to ketosis, acidosis, and inefficient caloric utilization.

With the advent of the Fischer-Tropsch and related syntheses, it became possible to consider synthesis of edible long-chain materials from CO_2 and H_2 . Much of the work was done in Germany during 1938-48, although some effort along these lines has been accomplished in other countries. A number of patents also exist.

Synthesis of Fatty Acids

The Fischer-Tropsch synthesis (ref. 35) was first accomplished in 1923 with the report that $\text{CO} + \text{H}_2$ at 100-150 atm and at 400° to 450° C was converted to oxygenated compounds and hydrocarbons when passed over alkalized iron turnings. This original "synthol" product was not particularly desirable commercially and efforts through the years led to more efficient catalysts and conditions for the production of hydrocarbons (ref. 1). The thermodynamics for this process are now well known and comprehensive literature is available. For the purpose under consideration here, it is fortunate that a high percentage of the product is straight-chain since the metabolism of long branched chain compounds is not well known.

The commercial production of soaps and small amounts of edible fats was described by Imhausen in 1938 (ref. 36). Hydrocarbons obtained from the Fischer-Tropsch process were oxidized by air to fatty acids consisting mostly of C_{10} to C_{22} chains. After purification and conversion to soap, the product was quite similar to natural soap in its properties. Apparently, large amounts of this material were produced and used during the war. Experiments on the production of edible fats from the synthetic fatty acids by condensation with synthetic glycerol were successful on a laboratory scale but no data were reported.

After the war, additional information concerning the production and use of synthetic edible fats appeared (refs. 37-42). More than 20,000 animal experiments were conducted with the synthetic fat. A variety of human studies were also performed. The material was used metabolically in exactly the same manner as natural fat even though the synthetic fat contained an appreciable amount of odd-chain length fatty acids (refs. 43-46) which, however, did give rise to small amounts of succinic acid in the urine (ref. 46). A major advantage of the synthetic fat over natural fat was its great resistance to rancidity since it did not contain any unsaturated fatty acids. In later production runs, distillation of the fatty acids resulted in decreased content of methyl-substituted long-chain fatty acids which were felt to be undesirable since they led to an elevation of the ether-soluble fraction in the urine of individuals consuming this synthetic fat. The presence of odd-chain length fatty acids in the diet is probably acceptable since these are degraded normally to propionic acid which has been shown to be readily reconverted to fat or oxidized via the TCA cycle (ref. 47).

The glycerol moiety of fats can be obtained relatively easily by synthetic processes and it is produced in large amounts industrially. The condensation between fatty acids and glycerol can be accomplished by heat alone or at lower temperatures by the addition of zinc dust.

Various modifications of this approach to the synthesis of fat have been reported. Thus, the direct production of fatty acids without the need for an intermediate oxidation step can be accomplished (ref. 48) by maintaining a high concentration of CO_2 and H_2 with a special K_2CO_3 -promoted sintered red iron oxide catalyst. The conversion level was about 93 percent with a fatty acid yield of 25 cc/m³ of $\text{H}_2 + \text{CO}$ converted.

The Russians apparently recover by-product fatty acid from hydrocarbon syntheses since it has been reported (ref. 49) that purification can be accomplished by successive treatments with alkali and H_2SO_4 followed by fractional distillation.

The Red Chinese have devoted effort to reproducing and improving the Imhausen process for fatty acid production. A report (ref. 50) concerning their studies of reaction variables indicates that they were able to obtain a 93 percent yield of fatty acids of which 60 to 70 percent was C_{15} - C_{20} acids.

Efforts are beginning here to determine which of the various methods for the synthesis of synthetic fat is most amenable to miniaturization and incorporation into a spacecraft.

REFERENCES

1. von Butlerow, A. M.: Bildung einer Zuckerartigen Substanz Durch Synthese. Ann., vol. 120, 1861, pp. 295-298.
2. Loew, O.: Weiteres über die Condensation des Formaldehyds. J. Prakt. Chem., vol. 34, 1886, pp. 51-55.

3. Fischer, E.; and Passmore, F.: Bildung von Acrose aus Formaldehyd. Ber., vol. 22, 1889, pp. 359-361.
4. Euler, H. V.; and Euler, A.: Ueber die Bildung von i - Aro-binoketose aus Formaldehyd. Ber., vol. 39, 1906, pp. 45-51.
5. Akerlof, G. C.; and Mitchell, P. W.: A Study of the Feasibility of the Regeneration of Carbohydrates in a Closed Circuit Respiratory System. Rep. no. 63143 (NASr-88), FMC Corp., J. Spacecraft, vol. 1, 1964, p. 303.
6. Fischer, E.; and Tefel, J.: Synthetische Versuche en der Zuckergruppe. 11. Ber., vol. 20, 1887, pp. 3384-3390.
7. Katzschmann, E.: Organic catalysts. XXVI. Formaldehyde Condensation as an Organic Autocatalysis. 3. Reaction of Formaldehyde With Acetol. Ber., vol. 77B, 1944, pp. 579-585.
8. Pfeil, E.; and Ruckert, H.: Formaldehyde Condensation. Formation of Sugars From Formaldehyde by the Action of Alkalies. Ann., vol. 641, 1961, pp. 121-131.
9. Loew, O.: Zur Condensation des Formaldehyds. Ber., vol. 39, 1906, pp. 1592-1593.
10. Loeb, W.: Cleavage of Sugars. I. Action of Zinc Carbonate on Formaldehyde Solutions. Biochem. Z., vol. 12, 1909, pp. 78-96.
11. Seyewetz, A.; and Gibello: Syntheses de Sucres à Partir du Trioxy-methylene et du Sulfite de Soude. Comp. Rend., vol. 138, p. 150; Bull. Soc. Chim. France 3, vol. 31, 1904, p. 434.
12. Lind, S. C.; and Bardwell, D. C.: Chemical Action at Gaseous Ions Produced by α -Particles IX. Saturated Hydrocarbons. J. Am. Chem. Soc., vol. 48, 1926, p. 2349.
13. Sinyan, Y. Y.: The Possibility of Physiochemical Synthesis of Carbohydrates in a Space. Problems in Spae Biology (USSR), vol. 3, 1964, p. 401.
14. Fischer, E.: Ueber die optischen Isomeren des Traubenzuckers, der Glucomsäure und der Zuckersäure. Ber., vol. 23, 1890, p. 2611.
15. Rudney, H.: The Utilization of L-Glucose by Mammalian Tissues and Bacteria. Science, vol. 92, 1940, p. 112.
16. Boyd, W. C.; and Matsubara, Skoji: Different Tastes of Enantiomorphic Hexoses. Science, vol. 137, 1962, p. 669.

17. Barber, G. A.; and Hassid, W. Z.: *In vivo* Oxidation of L-rhamnose and L-glucose by Higher Plants. Bull. Res. Council Israel, Sec. A, vol. 11, 1963, p. 249.
18. Yamaguchi, K.: Studies on the Absorption of Unnatural Monosaccharides and Their Fates After Being Absorbed. J. Biochem. (Japan), vol. 43, 1956, p. 399.
19. Corley, R. C.: Pentose Metabolism III. A Comparison of the Rates of Disposal of d-arabinose and -arabinose in the Rabbit. J. Biol. Chem., vol. 82, 1929, p. 269.
20. Rice, E. W.; and Roe, J. H.: A Study of the Metabolism of D-arabinose in the Rabbit. J. Biol. Chem., vol. 188, 1950, p. 463.
21. Koser, S. A.; and Saunders, F.: Fermentation of the d- and -forms of ribinose by Bacteria. Proc. Soc. Exp. Biol. Med., vol. 30, 1932, pp. 218-220.
22. Wool, I. G.: Incorporation of C¹⁴ From C¹⁴-Labeled Sugars into CO₂, Nucleic Acids and Protein by Isolated Rat Diaphragm. Am. J. Physiol., vol. 198, 1960, p. 649.
23. Stjernholm, R. L.; and Noble, E. P.: Carbohydrate Metabolism in Leukocytes. V. The Metabolism of Five Carbon Substrates in Polymorphonuclear Leukocytes of Rabbit. Arch. Biochem. Biophys., vol. 100, 1963, pp. 200-204.
24. Rummel, W.; and Stupp, H. F.: Resorption von D- und L-glucose durch den Darm *in vitro*. Med. Exp., vol. 3, 1960, p. 303.
25. Morgan, H. E.; Regen, D. M.; and Park, C. R.: Identification of a Mobile Carrier-Medicated Sugar Transport System in Muscle. J. Biol. Chem., vol. 239, no. 2, 1964, pp. 369-374.
26. Battaglia, F. C.; and Randle, P. J.: Regulation of Glucose Uptake by Muscle. IV. The Specificity of Monosaccharide-Transport Systems in Rat-Diaphragm Muscle. Biochem. J., vol. 75, 1960, p. 408.
27. Bihler, J.; Cavert, H. M.; and Fisher, R. B.: A Differential Effect of Inhibitors on Sugar Penetration into the Isolated Rabbit Heart. J. Physiol., vol. 180, 1965, p. 168.
28. Cori, C. F.; and Cori, G. T.: The Fate of Sugar in the Animal Body. VIII. The Influence of Insulin on the Utilization of Glucose, Fructose, and Dihydroxyacetone. J. Biol. Chem., vol. 76, 1928, p. 755.
29. Tsao, M. U.; Schwartz, E. L.; and Lowrey, G. H.: Metabolism of Dihydroxyacetone in the Intact Rat. Metab. Clin. Exp., vol. 11, 1962, p. 456.
30. Tsao, M. U.; Bernstein, I. A., and Lowrey, G. H.: Metabolism of C¹⁴-Dihydroxyacetone in the Intact Rat. Metabolism, vol. 11, 1962, p. 465.

31. Cori, C. F.; and Shine, W. M.: The Formation of Carbohydrate From Glycerophosphate in the Liver of the Rat. *Science*, vol. 82, 1935, p. 134.
32. Stohr, R.: Beiträge zur Kenntnis des Physiologischen Verhaltens der Triosen und ihnen nahestehen der Verbindungen. II. Vermehrung des Leberglykogens nach Verfütterung von Glycerinaldehyd. (The Physiological Behavior of Trioses and Closely Related Compounds. II. Increase of Liver Glycogen After Feeding of Glycerinaldehyde.) *Z. Physiol. Chem.*, vol. 206, 1932, p. 211.
33. Stohr, R.: Beiträge zur Kenntnis des Physiologischen Verhaltens der Triosen und ihnen nahestehen der Verbindungen. III. Muskelglykogen und Blutzucker nach Verfütterung von Methyl Glyoxal und Glycerinaldehyd. (Physiological Behavior of Trioses and Related Compounds. III. Muscle Glycogen and Blood Sugar After Feeding Methylglyoxal and Glycerinaldehyde.) *Z. Physiol. Chem.*, vol. 212, 1932, p. 85.
34. Johnson, V.; Carlson, A. J.; and Johnson, A.: Studies of the Physiological Action of Glycerol on the Animal Organism. *Am. J. Physiol.*, vol. 103, 1933, p. 517.
35. Storch, H. H.; Golumbic, N.; and Anderson, R. B.: The Fischer-Tropsch and Related Syntheses, John Wiley and Sons, Inc., New York, 1951.
36. Imhausen, A.: Untersuchungen und Seifen aus synthetischen Fettsäuren. (Soaps From Synthetic Fatty Acids.) *Kolloid-Zeitschrift*, vol. 85, 1938, pp. 234-246.
37. Bergner, K. G.: Synthetische Nahrungsmittel. (Synthetic Food.) *Deutsche Lebensmittel-Rundschau*, vol. 43, 1947, pp. 2-6. Also NASA TT F-9895, 1966.
38. Imhausen, K. H.: Die Speisefett-Synthese. (Synthesis of Edible Fats.) *VDI-Zeitschrift*, vol. 91, 1949, pp. 463-467. Also NASA TT F-9894, 1966.
39. Schiller, G.: Synthetische Fette auf der Grundlage von Paraffinfettsäuren, Zusammenfassender Bericht, besonders über die chemischen und technischen Fragen. (Synthetic Fat From Paraffin Fatty Acids. Review of Technical and Chemical Problems), *Z. Lebensm.-Untersuch. u. -Forsch.*, vol. 88, 1948, pp. 174-190.
40. Wachs, W.; and Reitstötter, J.: Zur Frage der Herstellung synthetischer Fettprodukte in Deutschland. (The Preparation of Synthetic Fats in Germany.) *Angewandte Chemie B.*, vol. 20, 1948, pp. 61-68.
41. Thomas, K.; and Weitzel, G.: Fat and Fat Metabolism. *Fiat Rev. German Sci., Biochemistry Pt. I*, 1939-1946, pp. 1-57 (Pub. 1947).
42. Flossner, O.: Synthetische Fette, Arbeitsgemeinschaft Medizinischer Verlage G.M.B.H., Johann Ambrosius Barth, Leipzig, 1948, 69 pp.

43. Thomas, K.; and Weitzel, G.: Uber die Eignung des Kunstfette aus Kohle als Nahrungsmittel. (Suitability of Synthetic Fat From Coal for Human Consumption.) Deut. med. Wochschr., vol. 71, 1946, pp. 18-21.
44. Flossner, O.: Nutritional and Physiological Investigation of Synthetic Fats. Ernahrung, vol. 8, 1943, pp. 89-93.
45. Appel, H.; Berger, G.; Bohm, H.; and Schiller, G.: Zur Kenntnis der Fette aus Fettsauren mit ungerader Kohlenstoffatomzahl. (Fats From Fatty Acids With Uneven Numbers of Carbon Atoms.) Z. Physiol. Chem., vol. 266, 1940, pp. 158-173.
46. Thomas, K.; and Weitzel, G.: Succinic Acid in the Urine After Eating Synthetic Fat. Z. Physiol. Chem., vol. 282, 1947, pp. 180-185.
47. Cady, P.; Abraham, S. ; and Chaikoff, J. L. : Relative Incorporation of the Various Propionate Carbons Into Fatty Acids by Lactating Rat Mammary Gland. Biochim. Biophys. Acta, vol. 70, 1963, pp. 118-131.
48. Worsham, C. H.; and Holder, C. H. (to Standard Oil Devel. Corp.): Fatty Acid Production in Hydrocarbon Syntheses. U. S. Pat. 2,521,436, Sept. 5, 1950. C. A. 44, 11069, 1950.
49. Levkovich, M. M.: Apparatus for the Recovery of Fatty Acids From Lye Wastes. Byull. Tekh. Ekon. Inform. Sovet. Narod. Khoz. Rostovsk Ekon. Admin. Raiona, 1958, no. 12, vol. 40. From Chem. Abs., vol. 55, 1961, p. 8895.
50. Ministry of the Petroleum Industry: Oxidation of Synthetic Soft Paraffin to Fatty Acids. Jan Liao Hsueh Pao, vol. 4, 1959, pp. 15-32. From Chem. Abs., vol. 53, 1959, p. 21631.

PROSPECTUS FOR CHEMICAL SYNTHESIS OF PROTEINACEOUS FOODSTUFFS*

Sidney W. Fox

Institute of Molecular Evolution,
University of Miami

In considering chemical synthesis of foodstuffs, we can find some encouragement in the fact that nearly all vitamins are obtained more economically by chemical synthesis than by isolation from biological material (ref. 1).

Among the foodstuffs, the nature of vitamins has been most conducive to commercial chemical synthesis by stepwise conversion. The vitamins, as is well known, provide their effects in very small, or trace, amounts. An adult human's entire annual requirement of 3-(4-amino-2-methylpyrimidyl-5-methyl)-4-methyl-5, β -hydroxyethylthiazolium chloride, that is, thiamine chloride or vitamin B₁, can be synthesized chemically and sold for a few cents. This requirement is, however, no more than 1 g, whereas the annual requirement for "essential" amino acids such as leucine and lysine is measurable in pounds.

In commercial earthbound production of such food factors, many chemical reactions are employed to produce one vitamin or one amino acid.

In chemical synthesis under the conditions which may be available 250,000 miles or more from Rahway, New Jersey or Midland, Michigan, what we believe is most needed is the simultaneous synthesis of all of the food components in as few, and as simply operated, reactions as possible. We would thus want to emphasize procedures of pansynthesis (ref. 2). For example, the practical goal for space nitrogen nutrition appears to be one of synthesizing all of the amino acids needed in the diet and others, and polymerizing them simultaneously into one protein-like polymer, rather than (a) producing amino acids one at a time and (b) coupling amino acids into peptides one at a time.

The prospect of such simultaneous syntheses assumes some reality, particularly from model studies of the terrestrial abiotic origin of protein. These latter are found to emphasize, as do the syntheses carried out by living cells, the simultaneous chemical production of many components.

Additional rationale for seeking our clues in studies of models of the first protein is the premise of the evolutionary position of food. According to this premise, the first organism arose from spontaneously synthesized chemical components of food. The first living cell and its descendants maintained and continued themselves with more of those components. The belief that the problem of food for the first cell was not very different from that of contemporary cells, including human cells, has its roots in the principle of the "unity of biochemistry," the fact that all cells have a common chemical ground plan.

*Supported by NASA Grant NsG-689.

What has been presented to this point are reasons for looking into what would otherwise seem to be hopeless. These considerations are not a basis for easy optimism. In this connection, let me quote N. S. Scrimshaw's remarks from a 1963 symposium (ref. 3):

"The central theme of the discussion has been that the many problems of space feeding are not trivial ones. Moreover, they have not been solved, nor is the solution readily at hand. Furthermore, there is not adequate work underway to assure their solution at any early date or at any date at all. It is up to us as professional biologists, nutrition specialists, and other life scientists to show initiative both in identifying and solving these problems."

These comments apply with full force to the problems of chemical synthesis of food for space travel and for nutrition on extraterrestrial bodies such as Teller (ref. 4) has emphasized for one year sojourns, as on the moon, for example. In particular, we do not know how many problems remain to be identified. We do know that a principal problem is that of the closed carbon cycle, and that deprivation of nitrogen can lead to anemia, hypoproteinemia, and physiological malfunction (ref. 5), and death.

From this point, I will attempt to summarize briefly how we analyze the nitrogen cycle segment of our knowledge, and will point out some of the salient problems.

AMINO ACIDS

The synthesis of protein or similar polymers, chemically or biologically, generally requires amino acids as intermediates. In the laboratory studies of origin of life-related substances, the simultaneous synthesis of α -amino acids has received more attention than the origin of any other kind of biochemical substance. Two of these processes are illustrated in table I. One is the synthesis of Miller (ref. 6), a process of historical interest. In these reactions, 4 of the 18 or 20 amino acids--glycine, alanine, aspartic acid, and glutamic acid--were produced by electrical discharge in methane, ammonia, water, and hydrogen. Also, some amino acids which do not occur in protein were found as products.

In the same table, the experimental results of Harada and Fox (ref. 7) are presented where amino acids were produced by passing ammonia, water, and methane as gases through beds of silica, heated to 950° or 1050° C, and hydrolyzing the products.

TABLE I. - AMINO ACID COMPOSITIONS^a PRODUCED THERMALLY IN THE PRESENCE OF SILICA AND BY ELECTRIC DISCHARGE

	Thermal synthesis			Electric discharge synthesis	
	Silica sand 950° C, percent	Silica gel 950° C, percent	Silica gel 1050° C, percent	Spark discharge, ^b percent	Silent discharge, b percent
Aspartic acid	3.4	2.5	15.2	0.3	0.1
Threonine	.9	.6	3.0	---	---
Serine	2.0	1.9	10.0	---	---
Glutamic acid	4.8	3.1	10.2	.5	.3
Proline	2.3	1.5	2.3	---	---
Glycine	60.3	68.8	24.4	50.8	41.4
Alanine	18.0	16.9	20.2	27.4	4.7
Valine	2.3	1.2	2.1	---	---
Alloisoleucine	.3	.3	1.4	---	---
Isoleucine	1.1	.7	2.5	---	---
Leucine	2.4	1.5	4.6	---	---
Tyrosine	.8	.4	2.0	---	---
Phenylalanine	.8	.6	2.2	---	---
α -NH ₂ Butyric acid	.6	---	---	4.0	.6
β -Alanine	? ^c	? ^c	? ^c	12.1	2.3
Sarcosine	---	---	---	4.0	44.6
N-Methylalanine	---	---	---	.8	6.5

^aBasic amino acids were not listed in the table because those amino acids were not fully studied. Some amino acid analyses of the thermal products showed peaks corresponding with lysine (or ornithine) and arginine.

^bRecalculated from the results obtained by Miller (ref. 6).

^c β -Alanine peak obscured by another unknown peak.

Most of the amino acids that occur in protein are obtained by heating. Except for β -alanine, none of those which do not usually occur in protein are found. Among the common amino acids not found in the hydrolysate are cystine, methionine, histidine, and tryptophan. So far, the sulfur-containing amino acids have not been identified in the few experiments in which simple sulfur-containing compounds have been included in the reactant gases. The origin of such amino acids represents one of the problems to be solved. The absence of tryptophan can be explained as due to its known lability during mineral acid hydrolysis for analysis. The absence of histidine from the analysis may be explained by the fact that histidine and ammonia have closely similar R_f values on the automatic analyzer chromatograms, and much ammonia is present, since it was a reactant.

In addition to the fact of a few missing amino acids, the yields are low. How low is the yield is not yet known, since such information requires special apparatus when vapor phase syntheses are employed. So far, such apparatus has

not been used. As in engineering practice, however, unreacted gases and unwanted products should be combustible to simple gases which can be cycled again through the silica column.

Abelson (ref. 8) showed that CO_2 could function in place of methane in the electrical synthesis of amino acids. This carbon source should also be tested in thermal synthesis.

The experiments with silica give the results indicated in the first three columns, whereas the products obtained from alumina, volcanic beach sand, and from heated empty tubes by Ponnampertuma and by Oró (ref. 9) resemble qualitatively the amino acid pattern from electric discharge. Different kinds of electric discharge and different temperatures in the thermal synthesis yield quantitatively different amino acid compositions. Accordingly, experimental ways of controlling the nature of the products can be visualized.

With an adequate supply of amino acids, two questions can be asked: (a) What can be learned about the requirements for amino acids in protein nutrition and (b) Can they be used directly for nutrition? Answers to these questions have been provided by many workers, particularly by Rose (ref. 10) who has devoted much of his scientific lifetime to feeding mixtures of pure, crystalline amino acids to several species. Rose taught us (1) which amino acids must be supplied in the diet for each of several species, (2) which can be synthesized by the organism, (3) that humans make little use of amino acids in the D form, and (4) much about the way in which the presence or absence of one amino acid influences the need for another. Rose and others have taught us also that the problems and processes of protein nutrition are more complex than these few examples of information indicate. Whether pure L-amino acids must be used is yet to be established. In fact, experiments by Van Pilsum and Berg (ref. 11) indicate that the mammal can accommodate D-amino acids as provided by racemates.

The use of free amino acids as foods, however, poses questions. Is the utilization less efficient than when amino acids are slowly released by the digestion to which man has adapted? Disturbances of ion balances in the blood can occur if amino acids enter too rapidly. Taste is also a problem. Attempts to supply protein nutrition by feeding free amino acids as protein hydrolysates after World War II were unsuccessful (personal communication between W. Griffith and G. Lewis, 1965).

Perhaps these and other problems can be solved. An alternative area of investigation, however, is that of combining amino acids into polymers with sufficient properties of protein so that they can be employed nutritionally instead of protein. Since humans have been adapted to proteins by both biochemical evolution (ref. 12) and culture (e.g., preparation of colloidal foods; proteins have some inimitable properties), and since the contained amino acids must be simultaneously available (ref. 13), the principal requirement appears to be that of simultaneous inclusion of all the "essential" amino acids in peptide bonds in polymers of amino acids.

Two ways of approaching this goal have now been indicated, and the nutritional potential for investigation and for feeding has begun. The two

methods of synthesis are the simple condensation polymerization and the more intricate synthesis through the Leuchs anhydrides (ref. 2).

THERMAL PROTEINOIDS

The thermal condensation of α -amino acids can be brought about by heating a mixture containing sufficient proportions of nonneutral amino acids such as aspartic acid, glutamic acid, or lysine (refs. 14 and 15) for several hours at temperatures above the boiling point of water, 170^o.

Under such conditions, some proportion of each of the amino acids common to protein is found in the polymer, which typically has molecular weights of many thousands. Other properties in common with protein are listed in table 11. In the evolutionary context, the tendency to form, on contact

TABLE II. - PROPERTIES OF THERMAL POLYANHYDRO- α -AMINO ACIDS IN COMMON WITH PROTEINS*

Qualitative composition
Quantitative composition
Range of molecular weight
Color tests
Solubilities
Inclusion of nonamino acid groups
Optical activity
Salting-in and salting-out properties
Precipitability by protein reagents
Hypochromicity
Infrared absorption maxima
Recoverability of amino acids on hydrolysis
Susceptibility to proteolytic enzymes
Catalytic activity
Inactivatability by heating in aqueous solution
"Nonrandom" (nonuniform) sequential distribution of residues
Nutritive quality
Morphogenicity

*Bibliography given in reference 16.

with water, microspherical particles with many of the properties of cells is particularly provocative. This follows since the processes of polymerization and spherule formation are simple enough and otherwise imputable to the geological realm. The fact that five laboratories have recently reported weak catalytic activities for conversion of various cellular substrates provides a conceptual basis for the origin of metabolism.

As will be shown, these polymers have some, at present limited, utility for nutrition. These materials are known as proteinoids (ref. 16) to indicate that (a) they resemble proteins and (b) they are not necessarily to be regarded strictly as proteins. The differences are mostly more quantitative than qualitative and possibly reside more in the structure than in the function. Known differences are listed in table 111. Some proteins are found

TABLE III.- DIFFERENCES IN COMPOSITION OF PROTEINOIDS

Thema 1 homopolylysine ^a		
Temperature of condensation, °C	α-lysine, percent	ε-lysine, percent
180	34	66
190	39	61
200	45	55
210	48	52
Thermal copolyanhydro (lysine, alanine) ^b		
200	27	73
210	35	65

^aK. Harada (Ph.D. thesis, 1961).

^bHarada and Fox, Arch. Biochem. Biophys. 109, 55 (1965).

to differ in their properties from the properties of protein in general. Likewise, the properties of some proteinoids differ from those of proteinoids in general. Probably the sharpest difference at present is that of antigenicity. Some proteins, however, are devoid or almost devoid of antigenicity. Also, although antigenicity has not yet been observed in proteinoids, reasons for making and testing other polymers for this property are at hand.

The manner in which some amino acids, particularly the trifunctional lysine, are linked into thermal polymers is sensitively related to conditions of condensation. In the condensation of lysine, for example, the proportion of this amino acid linked through the α-amino group and the proportion linked through the ε-amino group is a function of temperature (table 111). Also, in our laboratory F. Suzuki has shown that the composition, isoelectric point, and molecular weight of thermal copolymers of lysine with trifunctional aspartic acid or trifunctional glutamic acid vary with the individual dicarboxylic acid and with whether the free basic amino acid or its

hydrochloride is used in the synthesis. The nature of such products is also altered by employing glutamine instead of glutamic acid, and asparagine instead of aspartic acid.

In view of the observed effects of temperature, proportions of specific amino acid, the choice between free basic amino acid and its salts, the choice between dicarboxylic amino acid and its amide, and probably unidentified factors as well, systematic investigation of the control of linkage is in prospect. This kind of study is basic to incorporating rapidly digestible linkages. The evolutionary relationships suggest that the intrinsic reactive tendencies of the amino acids would lead, however, to the kinds of products which were the basis for food and for evolution. For example, in France, Biserte (ref. 17) has studied the basic amino acids--lysine, ornithine, and α, ϵ -diaminobutyric--for their tendency to condense thermally (fig. 1).

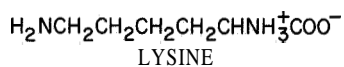
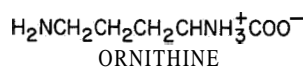
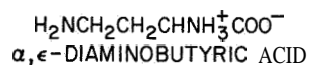


Figure 1.- Structures of dibasic amino acids.

Only lysine condenses thermally and it is the only one of the three found in protein or in food. This selectivity is consistent with the concept of limiting direction in evolutionary processes.

The relative proportions of amino acids in thermal proteinoids, such as 2:2:1- or 1:1:1-,¹ appear at first observation not particularly favorable for efficient space nutrition. In the past, moderately high proportions of aspartic acid or lysine in reaction mixtures were necessary to attain at least 10-percent yields of polymers of the kind that contain all 18 amino acids. In these cases, the proportion of aspartic acid or lysine in the corresponding polymers was above a level that was most effective for nutrition, being typically 50 percent, and well above the usual proportion of aspartic acid or lysine in food proteins.

Three adjustments to this state of affairs can be indicated. One of these was stated by Scrimshaw (ref. 18). Up to 80 percent of the dietary nitrogen can, he pointed out, be merely usable nitrogen, 20 percent being the amino acids essential for human diets--tryptophan, phenylalanine, threonine, isoleucine, lysine, methionine, valine, and leucine.

In a second approach to improving the balance of amino acids in the polymer, one could visualize condensing an intermediate proportion of aspartic acid and lysine each with other amino acids, instead of a moderately high proportion of one of these two. This experiment has recently been performed by Dr. Thomas Waehneltd in our laboratory and he has found that simple equimolar proportions of all reacting amino acids suffice. The analyses (table IV) show that proportions of these amino acids more closely resembling those of proteins can be attained. Since these results are recent, such polymers have not yet been tested nutritionally.

¹The figures 2:2:1- and 1:1:1- denote, respectively, parts of aspartic acid, glutamic acid, and an equimolar mixture of the 16 other amino acids in a reaction mixture.

TABLE IV. - ANALYSES OF NEUTRAL PROTEINOIDS (MOLE PERCENT)

Amino acid	Insoluble fraction	Soluble fraction
Lysine	5.6	7.1
Histidine	1.3	2.8
Arginine	2.8	3.3
Ammonia	21	15
Aspartic acid	2.8	3.9
Glutamic acid	5.9	8.7
Threonine	.3	.3
Serine	.1	.2
Proline	4.1	2.3
Glycine	7.2	7.1
Alanine	8.3	10.3
Valine	4.6	8.2
Methionine	5.7	5.8
Isoleucine	3.4	4.0
Leucine	5.5	5.1
Alloisoleucine	2.4	3.1
Cystine	3.7	4.4
Tyrosine	6.7	4.3
Phenylalanine	8.3	3.9

LEUCHS PROTEINOIDS

A third answer to the problem of proportions is to prepare Leuchs proteinoids containing all amino acids common to protein by a process based on the Leuchs anhydrides, or N-carboxyamino acid anhydrides of amino acids. The synthesis to include all proteinogenous amino acids has been accomplished with Dr. T. Hayakawa through the N-carboxyanhydrides of nine α -amino acids and of nine specially protected amino acids (ref. 2).

While the thermal proteinoids have the attractions of simplicity of preparation, and serve as a model of primordial protein, the Leuchs proteinoids contain amino acids which are slightly or not at all racemized and which seem not to pose questions of unusual linkages between amino acids.

To accomplish this synthesis, threonine was protected as the O-acetate; serine, cysteine, and tyrosine as the benzyl ethers; aspartic acid and glutamic acid as the β - and γ -benzyl esters, respectively; lysine as the ϵ -carboboxy derivative; arginine as the dicarboboxy derivative; and histidine as the ring-N-benzyl derivative. These substituting groups were substantially removed in a single operation, and were, in fact, chosen for such removability. However, the product is not easy to produce.

This was the first polymer which closely resembled on hydrolysis and analysis a natural protein.

Figure 2 shows the analysis of the hydrolysate of one of four Leuchs proteinoids prepared in Hayakawa's study. The composition was patterned

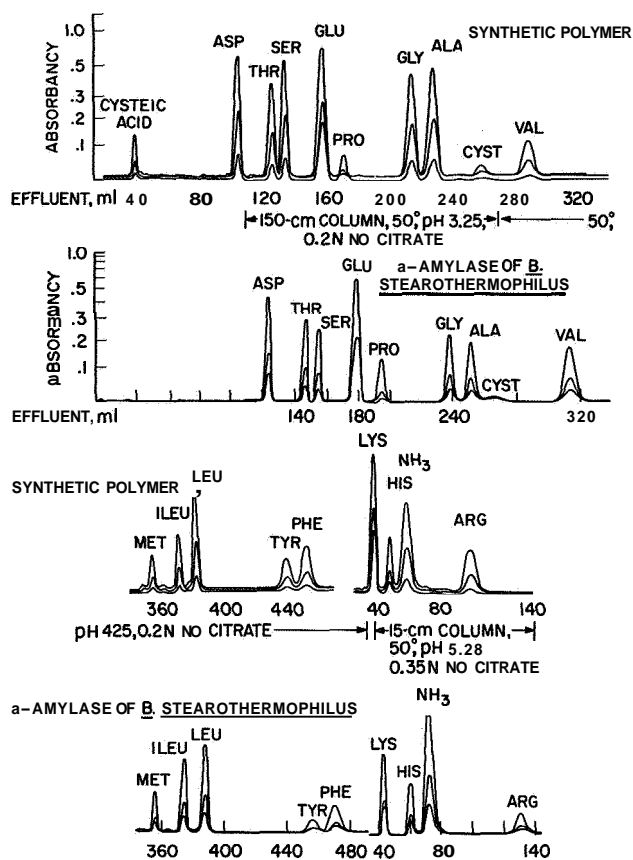


Figure 2.- Amino acid profiles of hydrolysates of a Leuchs proteinoid and of a-amylase from *Bacillus stearothermophilus*.

The first experiments were conducted some years ago with an assay organism *Lactobacillus plantarum*, which has amino acid requirements similar to those of man. The best thermal proteinoid available at the time of testing was roughly 60 percent as effective as peptone in supporting the growth of these bacilli. Other thermal proteinoids with a less favorable balance of amino acids yielded weaker nutritive response.

The experiments with rats have been done by Krampitz and his colleagues and have partly been published in several papers. Krampitz and Knappen (ref. 20) performed experiments in which radioactive methionine was thermally condensed with other amino acids and the water-soluble polymers were fed to rats. The distribution of this material in the protein and nonprotein fractions of various tissues of the rat are given in table V. Krampitz and co-workers have learned, by replacing half of the biological protein in a diet by thermal proteinoid and supplementing with threonine, that the rats will gain approximately the same weight as with the same total weight of feed protein (personal communication from G. Krampitz, 1966). No toxicity was observed in extended feeding (ref. 20).

after an average set of values obtained in a survey of compositions of protein. The meaning of average values for composition is to be judged in the context of the fact that compositions of heterologous proteins are known to be more similar than different. The first protein for which an automatic amino acid profile was found was the a-amylase of *Bacillus stearothermophilus* (ref. 19). The interdigitated profiles of the hydrolysates of the synthetic and natural polymers are presented and speak for themselves. The principal difference is the presence of cysteic acid in the synthetic polymer. Variations in the four polymers show that the composition is easily controlled.

NUTRITIVE PROPERTIES

The nutritive qualities that have been studied in proteinoids have been recorded for *Lactobacillus plantarum* and for two animals, laboratory rats and the protozoan *Tetrahymena pyroformis* R.

	Radioactivity recovered from carcass, percent		
	Nonprotein fraction	Protein fraction	Total
Blood	6.43	24.41	32.84
Skin and hair	---	---	12.08
Liver	7.68	1.44	9.12
Brain	5.72	1.90	7.62
Kidneys	1.87	1.61	3.48
Intestinal tissue	2.09	2.18	4.27
Rest of body	10.27	20.32	30.59

Working under the supervision of Professor George Lewis, Everett (ref. 21) studied the utilization of proteinoids by the protozoan Tetrahymena pyroformis R. The thermal proteinoids alone did not support growth, but they did support such growth when supplemented with free amino acids. Tetrahymena grow better with free amino acids than with proteins. However, in figure 3, the equimolar

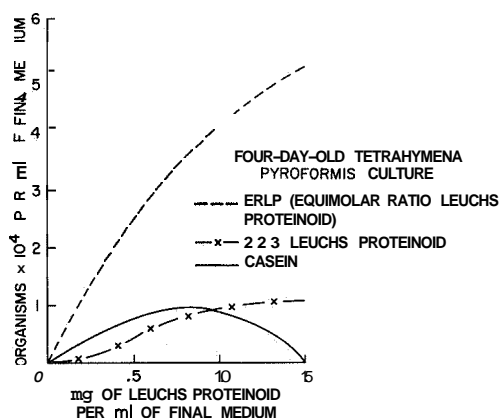


Figure 3.- Growth of Tetrahymena pyroformis on Leuchs proteinoids.

ratio Leuchs proteinoid is more effective than that patterned after a (2:2:3) thermal proteinoid which is about 40 percent aspartic acid. Therefore, it has a much more favorable balance of amino acids. Casein is not a suitable control for this organism. The possibility of supernutritional Leuchs proteinoids can be visualized, however, on the basis that a better amino acid balance can be found than in bioprotein.

This paper has indicated basically some of the gaps that must be filled for the objectives stated. The favorable aspects of knowledge from studies of

molecular evolution have been pointed out, particularly the rationale of pansynthetic approaches. Some of the results with pansyntheses have been demonstrated. Another favorable development not discussed is the existence of automatic peptide synthesizers, designed by Merrifield (ref. 22). Some problems have not been discussed, for example, incineration chemistry and palatability. (The proteinoids have tolerable flavor.) Other problems have been partially analyzed. Others remain to be identified. The studies of free amino acids and of protein-like polymers appear to be more balanced than the ones on biological sources at a comparable stage of development. We draw this conclusion from the fact that nutritional and toxicity studies and observations of taste were performed, in each case, before engineering analysis. Even so, much of what has been presented is recent and incomplete. The most meaningful summary at this point is perhaps the realization that a new door, the chemical pathway, to investigation in space nutrition is open.

REFERENCES

1. Fox, S. W.: The Outlook for Synthetic Foods. *Food Technol.*, vol. 17, 1963, pp. 388-392.
2. Fox, S. W.; Harada, K.; Krampitz, G.; Hayakawa, T.; and Windsor, C. R.: Chemical Synthesis of Proteinoids: Part I. NASA SP-70, 1964, pp. 331-338.
3. Scrimshaw, N. S.: Remarks in Panel Discussion on Food and Nutrition Problems in Prolonged Space Travel. *Federation Proc.*, vol. 22, 1963, p. 1459.
4. Teller, E.: Water Generation in Space. NASA SP-70, 1964, pp. 175-178.
5. Yoshimura, H.: Adult Protein Requirements. *Federation Proc.*, vol. 20, Suppl. 7, 1961, pp. 103-110.
6. Miller, S. L.: Production of Some Organic Compounds Under Possible Primitive Earth Conditions. *J. Am. Chem. Soc.*, vol. 77, 1955, pp. 2351-2361.
7. Harada, K.; and Fox, S. W.: Thermal Synthesis of Natural Amino-Acids from a Postulated Primitive Terrestrial Atmosphere. *Nature*, vol. 201, 1964, pp. 335-336.
8. Abelson, P. H.: Amino Acids Formed in "Primitive Atmosphere." *Science*, vol. 124, 1956, p. 935.
9. Orb, J.: Stages and Mechanisms of Prebiological Organic Synthesis. *The Origins of Prebiological Systems*, S. W. Fox, ed., 1965, pp. 137-171.
10. Rose, W. C.: The Amino Acid Requirements of Adult Man. *Nutr. Abstr. Rev.*, vol. 27, 1957, pp. 631-647.
11. Van Pilsum, J. F.; and Berg, C. P.: The Comparative Availabilities of Mixtures of the L- and DL-Modifications of the Essential Amino Acids for Growth in the Rat. *J. Biol. Chem.*, vol. 183, 1950, pp. 279-290.
12. Fox, S. W.: Experiments Suggesting Origins of Amino Acids and Proteins. *Protein Nutrition and Metabolism*, J. Kastelic, H. H. Draper, and H. P. Broquist, eds., Univ. of Illinois, College of Agriculture, Urbana, Illinois, Special Pub. No. 4, 1963, pp. 141-154.
13. Cannon, P. R.: The Problem of Tissue Protein Synthesis. *Federation Proc.*, vol. 7, 1948, pp. 391-396.
14. Fox, S. W.; and Harada, K.: The Thermal Copolymerization of Amino Acids Common to Protein. *J. Am. Chem. Soc.*, vol. 82, 1960, pp. 3745-3751.

15. Fox, S. W.; Harada, K.; and Rohlfing, D. L.: The Thermal Copolymerization of α -Amino Acids. Polyamino Acids, Polypeptides, and Proteins, M. Stahmann, ed., Univ. of Wisconsin Press, Madison, 1962, pp. 47-54.
16. Fox, S. W.: Experiments Suggesting Evolution to Protein. Evolving Genes and Proteins, V. Bryson and H. Vogel, eds., 1965, pp. 359-369.
17. Fox, Sidney W.: Comments on A Colloquium on Elementary Biological Systems and Biogenesis, Paris, France, November 23-25, 1965. Bioscience, vol. 16, no. 7, July 1966, pp. 480-481.
18. Scrimshaw, N. S.: Comments in NASA SP-70, 1964, p. 338.
19. Campbell, L.; and Manning, G. B.: Thermostable α -Amylase of Bacillus stearothermophilus. J. Biol. Chem., vol. 236, 1961, pp. 2962-2965.
20. Krampitz, G.; and Knappen, F.: Chemical Synthesis of Proteinoids: Part II. NASA SP-70, 1964, pp. 339-341.
21. Everett, P.: The Nutritional Value of Thermal and Leuchs Proteinoids Using Tetrahymena pyroformis Strain W as Assay Organism. M. S. Thesis, Univ. of Miami, 1965.
22. Merrifield, R. B.: Quoted in Chem. Eng. News, June 21, 1965, p. 40.

THE EFFECTS OF CONTROLLED ENVIRONMENT ON THE GROWTH OF
HYDROGENOMONAS BACTERIA IN CONTINUOUS CULTURES

By John F. Foster and John H. Litchfield

Battelle Memorial Institute
Columbus Laboratories

The continuous culture of Hydrogenomonas is the intermediate step of a three-step closed life support cycle directed to the conversion of the human metabolic wastes, urea and carbon dioxide, into breathable oxygen and a food supplement. The potential advantages of this scheme have been discussed in an earlier paper (ref. 1). The other two steps of the complete cycle are: (1) the collection and recovery of the CO₂ removed from the cabin atmosphere by a regenerable CO₂ absorber, so that relatively pure CO₂ gas can be fed into the gas phase of the continuous culture; and (2) the electrolysis of water to produce breathable oxygen for the cabin supply, and by-product hydrogen, which is also fed to the gas phase of the closed culture vessel. There are three specific advantages in the use of this culture: (1) the bacteria use waste urea as a sole nitrogen source during growth, together with the CO₂ waste as a carbon source; the harvested excess of cells from a steady-state culture is a potential food for man; (2) the organism is not photosynthetic, so the equipment designer need not confine the culture in thin layers to have light available to all parts as is necessary in photosynthesis; and (3) the culture uses the by-product hydrogen from electrolysis as an energy source. Some oxygen must also be supplied to the culture environment, because the energy of the growth process is derived from the metabolic hydrogen-oxygen reaction, with the production of water that can be recycled to the electrolysis cell.

OBJECTIVES

In developing a workable closed cycle as outlined, the primary objective is to control the environment of the continuous culture so as to maintain a steady state indefinitely, operating at the highest efficiency. The highest overall efficiency is an optimum combination of three separate efficiencies whose relative importance may vary with the requirements placed on the culture by the space mission in which it forms a part of the life support system.

The individual efficiencies are: (1) CO₂ conversion efficiency, qualitatively defined as the ratio of CO₂ conversion rate to hydrogen and oxygen conversion rate; (2) urea conversion efficiency, arbitrarily and qualitatively defined as the ratio of protein harvested in the cells to the

urea converted; and (3) the rate of production of harvested cellular material potentially useful as food per unit volume or weight of the continuous culture in its steady state.

Two other particular objectives are regarded as secondary but are important during this phase of the research studies: the maximum production of harvested cellular material for use in feeding studies to determine its applicability as a human food supplement, and the development of reliable, efficient, and accurate apparatus in which the continuous culture can be studied. It is anticipated that the ultimate design of such equipment would be a reliable guide for the design of flight-type apparatus for use on a mission.

EQUIPMENT CHARACTERISTICS

The design principles, development and construction, and first operation of the original continuous culture equipment have been described (ref. 1), and the modified equipment presently in use still operates on the same basic principles. The quantitative value of each of the controlled variables in the environment of the continuous culture is monitored by individual sensors whose signal is a specific function of the concentration or level of that one variable. At the present time, these variables include the following: partial pressures of the gases CO_2 , hydrogen, and oxygen; hydrogen ion concentration as pH; urea concentration; cell population density measured as optical density at 650 μ ; temperature; and total pressure. The signals from the sensors for the variables under automatic control are scanned in sequence, each one during a 10-second interval in the total time cycle of 1 minute. These signals are carried to a recorder-controller and recorded in the same sequence on a single chart. An individual control circuit is assigned to each recorded signal, with the circuit set to demand an addition of the appropriate material at a preset minimum concentration. The principle of incremental additions is used, in which the demand signal triggers the addition of a fixed and preset amount of the deficient component in the environment. During the next minute, the concentration is again examined, and another increment is added if demanded. When the deficiency is satisfied, the concentration is recorded but nothing is added until concentration again falls below the minimum. Some departures from these basic principles of control have been required by the limitations of the available equipment, but the effective operation is the same as described here. There are or have been as many as five different charts of simultaneous recordings of culture environment, which are correlated on a real-time scale in interpreting the data. As convenience, cost, or availability may permit, recording and controlling functions are combined, or added, or redundancies are eliminated with continuing development of the apparatus.

QUALITATIVE OBSERVATIONS ON ENVIRONMENTAL EFFECTS

Since there had been no previous experience in the experimental continuous culture of Hydrogenomonas eutropha, the immediate objective of each experiment during the past two years of experimental study has been to achieve a high growth rate at high cell density, and to maintain these in a steady state for a significant length of time. Identification of the controlling variables that produced favorable or unfavorable trends was attempted after each experiment to guide the next experiment. This required selective interpretations and tentative conclusions based on all accumulated experience.

There have been evidences in the course of the continuous-culture studies of gratifying progress in the form of improved equipment reliability and in better precision, completeness, and significance of environmental data. With this progress, the accumulating observations of environmental effects have shown certain regularities in performance of the growing cultures. These are reviewed in this section as background for the latest studies on urea assimilation, which are described for the first time in a following section.

Gas Assimilation Rates

Bongers (ref. 2) has pointed out that the conversion of CO_2 by Hydrogenomonas eutropha at maximum efficiency should assimilate the three environmental gases in the ratio of 6 H_2 :2 O_2 : CO_2 . Lower efficiencies would require relatively more hydrogen and oxygen per mole of CO_2 converted, such as, for example, a gas consumption ratio of 8 H_2 :3 O_2 : CO_2 . The assumptions used in calculating these ratios are overly simplified because the conversion of urea to protein by the culture is an important part of the total process in which CO_2 is assimilated. Nevertheless, it has been observed that the gases are usually assimilated in ratios close to the 6:2:1 combination. This ratio pattern seems to deviate largely only when a major departure from a tolerable environment occurs. Then the rate of CO_2 conversion to cell carbon may slow down considerably more, relatively, than the rates of consumption of H_2 and O_2 .

The total gas consumption rate exhibits a comparatively sensitive and rapid response to a change in environmental conditions, especially when the change is favorable to an increase in the growth rate of the organism. In a number of instances an increase in gas consumption rate is definitely measured within 30 minutes after an environmental change, whereas it may be several hours before the trend can be detected directly as an increase in growth rate.

There appears to be a favorable range of about 5 to 8 percent CO_2 in the gas phase, which enhances culture growth. The partial pressure of CO_2 appears to interact with other variables in the system, particularly pH, and possibly urea concentration, in ways that are not yet quantitatively clear. Therefore, the favorable CO_2 range may differ as other variables change. It has been increasingly clear, in repeated instances following an abrupt change

in the culture environment, that the resulting change in the CO_2 consumption rate lags behind the relatively rapid changes in hydrogen and oxygen consumption rates by about 4 hours, or about one generation time period of the organism.

Bongers (ref. 3) has noted an inhibiting effect of high partial pressures of oxygen on growth in batch cultures. In these continuous-culture studies, we have attempted to avoid high oxygen concentrations in the gas phase. A range of 7 to 10 percent oxygen has apparently been acceptable. In some cultures an excursion to high values of 25 percent or more oxygen has occurred inadvertently because of a deteriorating signal from an overage oxygen sensor in the control system. Nevertheless, the sluggish behavior of a culture in an aberrant environment could not be clearly identified as oxygen inhibition of growth.

There have been no identifiable effects of hydrogen partial pressure because of the minor fluctuations around the median value of about 85 percent hydrogen in the gas phase, which has been used in all the continuous culture experiments.

Hydrogen Ion Concentration

Active growth of continuous cultures has occurred in the approximate pH range of 6.2 to 7.0. At pH values above the upper limit, it is probable that shifts in the CO_2 -bicarbonate-carbonate equilibria in the liquid phase may reduce the assimilation of CO_2 by the organism and thereby inhibit growth. When the pH of the liquid phase is below 6.2, the mode of growth appears to change unfavorably. However, the trend toward low pH appears to be a result of an unfavorable growth environment caused by other variables, rather than an independent cause of poor growth. It has been observed that a stable pH in the favorable range is characteristic of a stable steady state. Cultures in a very active growth mode (which is desirable) tend to cause a pH rise in the medium.

An automatic pH recorder-controller was added to the system only recently to study, in more detail, pH variations and levels whose influences were inferred, as generalized above, from intermittent manual sampling and pH measurement during the work day only. The automatic control was set to add hydrogen ion as HCl solution at the control point of $\text{pH} = 6.7$ to counteract rising pH during a rapid growth condition in the continuous culture. Such pH control was not sufficient or appropriate to extend the active growth mode indefinitely, which reinforced the tentative conclusion above that a trend in pH away from a stable value is an effect and not a cause of changing growth activity. The further inference is that other variables, such as CO_2 partial pressure and urea concentration, are controlling growth. Feedback from the pH signal may be useful in future experiments to control these primary variables and thus to maintain rapid growth.

Cell Density and Growth Rate

The population density of cells in the liquid phase should be as high as possible to attain the highest volumetric efficiency of the culture apparatus. Simultaneously, the growth rate of the dense cell population must be maintained at a maximum level to convert the waste-product inputs into a useful harvested material as rapidly as possible. On the basis of reports from Bongers (unpublished communications) that cell densities higher than 10 g (dry weight)/liter were obtained in batch cultures, a tentative goal of 10 g/liter has been set for the study of dense continuous cultures of high growth rate.

A goal of 0.26 per hour for the growth rate constant was also set for dense continuous cultures. This value was selected because it is the approximate maximum growth rate observed repeatedly in the logarithmic growth phase of batch cultures prior to reaching the cell density desired for steady-state operation. A similarly high growth rate has also been achieved for relatively short periods of several hours during continuous culture growth following a change from one set of environmental conditions to another.

So far, these goals have not been met. There is real optimism now that they can be achieved, and perhaps exceeded substantially, because of a new and much broader understanding of growth mechanisms being developed in the current series of experimental cultures. The outlines of these growth mechanisms are discussed in the final section of this paper.

Up to the present time, a cell density of about 7 g/liter has been demonstrated in a continuous culture growing at a rate of about 0.22 per hour. A growth rate of about 0.23 has been reached in cultures with about 4 to 5 g of cells per liter, but this rate could not be maintained continuously in one experiment when an increase in the cell density to a higher value was attempted.

Composition of Harvested Cells

The culture environment influences the elementary chemical composition of the cellular material. In particular, cellular nitrogen appears to correlate directly with the concentration of nitrogen (as urea) in the liquid growth medium. Analyses have shown about 14 percent nitrogen in harvested material is obtained with 2 to 3 g of urea per liter of medium. At an intermediate urea concentration of about 0.9 g/liter, the analyses show slightly over 12-percent elemental nitrogen. In a urea-deficient medium with 0.2 g or less urea per liter, the elemental analysis has shown about 10.7 percent nitrogen, and growth appears to be inhibited by nitrogen deficiency.

Protein, amino acids, DNA, and cell viability have been determined by manual methods on numerous individual and composite samples removed from the continuous cultures. A massive amount of raw data is available but it has not yet been quantitatively evaluated. Qualitative examination suggests that DNA content of the cells is a sensitive indication of growth activity and

culture environment. Nitrogen and protein determinations indicate that the harvested cells contain about 50 percent protein, with all the essential amino acids present.

Feeding tests have been drastically limited by the small amounts of harvested material that can be supplied. Calloway and Margen (ref. 4) report, on the basis of 1-week feeding tests with 10 weanling male albino rats (plus 10 controls), that: "Digestibility (or, more properly, net absorption) of H. eutropha nitrogen was slightly below that of casein--93 versus 99 percent--but within the normal range" Although the feeding data are too few to be decisive, these results are most encouraging.

Urea Assimilation

In all experiments preceding the last few, which are discussed later, analyses of the urea in the culture were made manually by the method of Coulombe and Favreau (ref. 5) from small (25 ml) samples withdrawn periodically (usually hourly during the work day) from the culture. Samples were stored frozen until the next day, when all were analyzed. The results appeared erratic, but careful review of the procedures and multiple parallel determinations on aliquots of the same sample indicated that storage practice and the analytical manipulations were quite acceptable. The accumulating results reinforced the early indications that the amount of urea added to the growing cultures in intermittent doses did not bear a simple relationship to the urea detectable before and after by analysis of the liquid phase. It seemed, qualitatively, that a part of the added urea was sequestered in some manner almost immediately. In two instances, detectable urea increased above the concentration level at which it had been measured soon after a large dose, when a second analysis was made an hour or so after the first, without any intervening second dose of urea.

Often the growth rate of a culture deteriorated when slow and continuous additions of urea were first started after a period of batch culture. These additions were necessary to replace the urea being consumed during the preliminary phase of a steady-state culture in which the cell density was being increased to the target value for the proposed steady state. The general impressions were that there are six features of urea assimilation: (1) a pseudoreaction in which new urea is sequestered by the culture, (2) an inhibiting effect of new urea on growth of the culture, (3) a time lag of several hours before the culture adjusts to a change in environment, and then a short period of shifting to a different steady state, (4) slow growth with possible formation of by-products at urea concentrations greater than about 1 g/liter, (5) slow growth because of nitrogen deficiency at concentrations less than about 0.2 g urea/liter, and (6) a relatively narrow range of urea concentration for optimum growth within the limits 0.2 and 1.0 g/liter. The real optimum probably depends upon the absolute values of other variables, such as CO₂ partial pressure, pH, and steady-state cell density.

The above impressions are an accumulation of partial interpretations from many experiments. Quantitative data were often not exactly reproducible,

and the above qualitative interpretations were not always possible for any one experiment because of interactions among the many variables in the culture environment. The inescapable conclusion was that continuous automatic analysis of urea concentration {ref. 6) must be applied to the culture together with feedback control at a preset urea level. The following section is a progress report describing parts of the first experiment with complete control of urea concentration. Some of the earlier interpretations are supported by the new data, but others require further study of the new data, as well as additional experiments.

UREA CONSUMPTION RATES

Figure 1 is a reproduction of a section of the recorder chart in which the upper curve represents urea concentration and the lower curve, cell density in the culture, both as functions of real time along the abscissa.

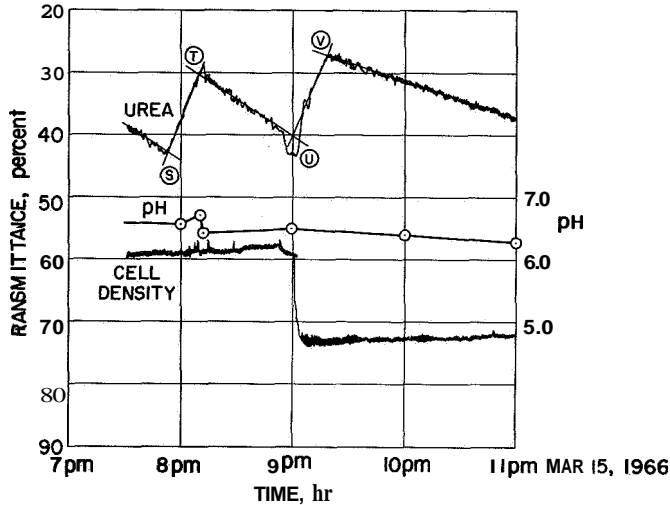


Figure 1.- Urea study - 7 p.m. to 11 p.m.,
March 15, 1966.

of urea is being added because urea is supplied more rapidly than it is consumed by the organisms. After urea input ceases, the urea concentration decreases as consumption by the organisms continues. The curve segments are approximately linear, and their positive or negative slopes are defined quantitatively by plotting an average straight line directly on the chart. The intersections of these straight lines correlate with the recorded times at which the automatic equipment started and completed each period of urea addition. These intersections are designated by capital letters entered on the charts so that segments of the urea curve can be identified by the two letters at the ends of each segment.

The ordinate on the left is percentage transmittance of the analytical samples, at 480 $m\mu$ for urea and 650 $m\mu$ for cell density. Calibration curves translate concentration in gravimetric units. The pH of the culture was recorded on another chart. Points from that original record were transferred to figure 1 to construct the middle curve. Some detailed features of the original record, as shown, are the subject of this discussion.

The sawtooth curve of urea concentration results from intermittent additions of urea solution to the continuous culture. The urea content rises while the concentrated solution

Urea Balance Calculations

The rates of urea consumption during each segment of the curve have been calculated by a urea balance applied to each segment. Table I shows a sample calculation for segment ST of figure 1.

TABLE I. - UREA BALANCE

Clock time: S, 7:50 p.m.; T, 8:06 p.m.; $\Delta t = 0.267$ hr Basis: One liter of culture	
<u>Urea in</u>	
Inventory at S, g/liter	0.65 ₇
Urea added by injector, g/liter	.19 ₆
Urea added with nutrient, g/liter	<u>.01₂</u>
Total	.86 ₅
<u>Urea out</u>	
Urea in overflow to harvests, g/liter	.01 ₂
Inventory at T, g/liter	<u>.80₁</u>
Total	.81 ₃
Urea consumed, by difference	.05 ₂
Consumption rate, g/liter-hr, $0.05_2/0.26_7$.20

Estimated Error

The calibration of the transmittance of the spectrophotometer cell with standard urea solutions gives a straight line on a semilog plot that defines the urea concentration within about ± 0.004 g/liter as read from the recorder chart. The intersections of the plotted positive and negative slopes of two consecutive curve segments resolve the time period of each segment to ± 1 minute on the recorder chart. The calculated rate of urea consumption for concentration differences and time periods of the magnitude represented by the urea balance of table I probably represents an uncertainty no larger than ± 0.03 g/liter-hr.

Further experiments will be necessary to eliminate the possibility of systematic errors and establish reproducibility. The data and discussion in the following sections are offered as a progress report, subject to possible revision after more data are obtained and evaluated.

Differences in Apparent Consumption Rates

Urea consumption rates have been calculated for a number of segments of the urea concentration curve by the method of urea balances described above in table I. Table II presents the results of these calculations, which include some parts of the continuous culture record that are not reproduced for this paper. For convenient reference, the urea rates are in two columns, giving apparent assimilation rates with and without additions of makeup urea to the culture.

TABU II.- APPARENT UREA CONSUMPTION RATES

Curve segment	Time span, hr	Urea rate, g/liter-hr	
		During urea addition	No urea addition
MN	0.25		0.17
NO	.68		.17
OP	.33	0.33	
PQ	.97		.16
QR	.32	.31	
RS	1.07		.13
ST	.27	.20	
TU	.83		.13
UV	.32	(.39)	
VW	2.47		.07
WX	.18	(.08)	
XY	2.52		.06
YZ	.28	.21	
ZAA	1.93		.10
AABB	.28	.25	
BCC	2.00		.09
CCDD	.27	.21	
DDEE	1.55		.08
EEFF	.48		.10
FFGG	.30	.12	
GGHH	.68		.08
HHII	.37	.14	
IIJJ	.48		.07

It is notable that the apparent rates are always higher during the periods of urea addition as compared with the intervening periods while the culture is assimilating urea from the inventory in the medium and the concentration is decreasing. It is inconceivable that the rate of urea assimilation could change abruptly at the beginning and end of each period of urea addition. A possible explanation is that there may be a rapidly reversible equilibrium between dissolved urea detectable by analysis and some form of absorbed urea sequestered on the cell surfaces. Presumably more urea would be absorbed as the concentration rises during addition periods, and some

would be desorbed to the solution as the concentration falls without additions. Such a hypothetical process would cause the observed differences in calculated rates, even if the metabolic assimilation rate remains approximately constant during short periods of alternate adsorptions and desorptions.

A slower change in consumption rates is indicated in the time span of 4 hours including MN to ST at the top of table 11. At time M the urea concentration was raised from a lower value to the target value of about 0.75 g/liter selected for this experiment. There followed a detectable decrease in apparent rates over the next 2 or 3 hours, which might not have been recognized as possibly significant were it not for qualitative evidence from earlier experiments that high concentrations of urea may inhibit growth or change the metabolic assimilation process. Perhaps 0.75 g urea/liter is above the optimum concentration range. Experiments at lower concentrations are planned.

Referring again to figure 1, the cell density curve at 9:00 p.m. reflects the abrupt decrease in cell population density caused by withdrawing half the active culture and then diluting the remaining culture to the normal volume of 2 liters by adding 1 liter of medium. The anomalous urea rates for segments UV and WX in table II are enclosed in parentheses. They are presumed to be unreliable because of the disruption during dilution of the automatic continuous urea analysis. The urea rates during VW and XY are about half those before dilution, which might be expected with only half as many growing cells per liter after dilution.

Following urea addition during CCDD, the urea injection apparatus was adjusted to add urea more slowly, so that the concentration would not overshoot as far above the set point before the analytical apparatus could respond by cutting off the injection. The qualitative effect was to reduce the apparent consumption rate during injection periods HGG and HIII. This result is in accord with the hypothesis that the amount of adsorbed urea increases with increasing concentration in the medium.

GROWTH RATE

During the whole time covered in figure 1 and for a preceding period after 2 p.m. of the same day, the culture was being diluted at a constant rate of 0.12 hr^{-1} . The cell density was slowly increasing, showing that the growth rate was slightly higher than the dilution rate. The pH was also drifting slowly upward. This drift was compensated occasionally by automatic addition of acid whenever the preset limit of $\text{pH} = 6.7$ was exceeded. Such an addition occurred at 8:10 p.m. as shown on the chart by an abrupt change from $\text{pH} = 6.7$ to $\text{pH} = 6.4$. It has been observed several times that an upward trend of pH is a qualitative indication of a relatively active continuous culture, at least in the environments studied experimentally so far.

Following the abrupt dilution of the culture at 9 p.m., the pH trended downward and growth rate slowed, for reasons not yet understood. Figure 2 shows these trends during a following interval, which is not quite consecutive, because a 30-minute interval has been deleted from the chart between figures 1 and 2. It is easily seen on figure 2 that the growth rate decreased to such an extent that it fell behind the dilution rate, and the cell density was dropping at midnight. Simultaneously, the change toward a more acid culture accelerated.

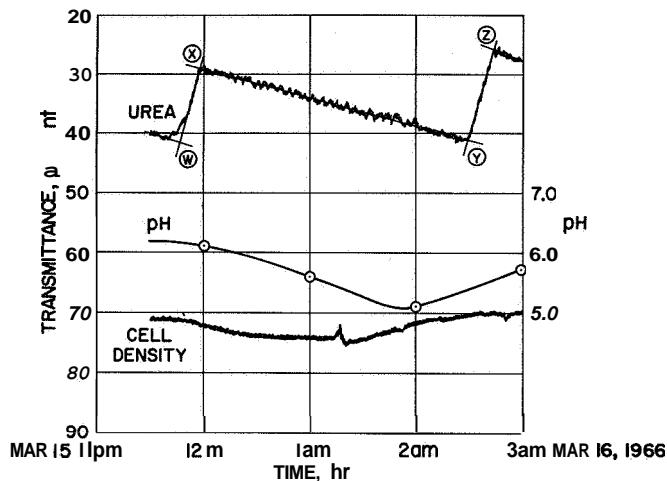


Figure 2.- Urea study - 11 p.m., March 15, 1966, to 3 a.m., March 16, 1966.

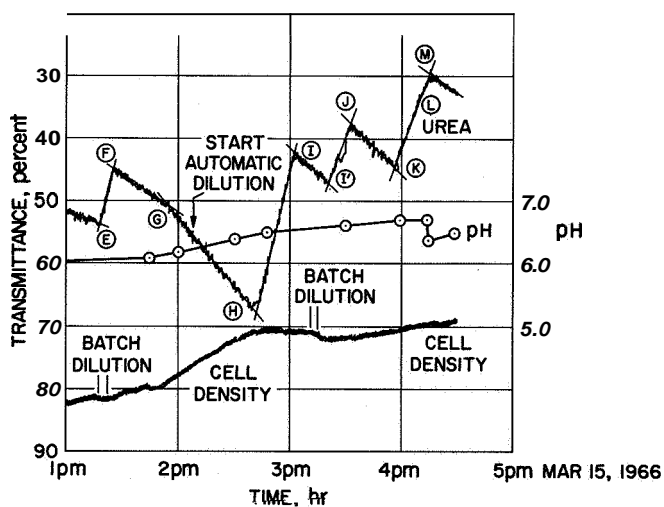


Figure 3.- Urea study - 1 p.m. to 5 p.m., March 15, 1966.

A spontaneous accommodation to culture environment appears at about 12:30 a.m., when growth matches dilution and cell density stabilizers. At 1:20 a.m. a small but rapid increase in transmittance occurs in the cell density detector, which would normally be interpreted as a decrease in cell density. Although the apparatus was unattended, the records indicate that all apparatus was operating properly, and that the change must have been a shift in the transmittance of the culture without a change in the cell population. This was followed immediately by an increase in growth rate, and about 40 minutes later by a turn-around in the pH trend toward increasing pH values. These are the recognizable characteristics of an actively growing culture. One other rapid change in the continuous culture had been the dilution 4.3 hours earlier, shown in figure 1. It seemed possible that the two were related, and that an induction period of about one generation time had intervened between cause and final effect.

Other parts of the recorded cell-density curve were reexamined for rapid changes similar to the one discussed above. Figure 3 shows a section of the chart record from early the preceding afternoon, which contains four small rapid reversals in the cell density. Two, at 1:20 p.m. and 3:12 p.m., were real reductions in cell density caused by batch dilutions to correct low culture volume caused by excessive discharge through the harvest

overflow. A third at about 4:15 p.m. was probably related to the change in pH following an acid addition at that time. A fourth at 1:45 p.m. could not be related to any simultaneous occurrence; however, it was followed by the increase in growth rate and the upward pH trend characteristic of the similar change in transmittance at 1:20 a.m., as described above. Furthermore, at 10:00 a.m., 3.8 hours before, one liter of the culture had been withdrawn and the remainder had been diluted 1:1 by adding a liter of medium.

It thus appears that some features of the culture environment exert an influence on culture characteristics after a delay period of about four hours. These influences are not yet understood or explainable, but it is probable that their existence must be recognized and accommodated before optimum growth can be attained, maintained, and controlled.

CONCLUSION

A large number of variables must be controlled in a continuous culture of Hydrogenomonas eutropha and these apparently interact to cause nonlinear responses in a control system. The development of a better understanding of the control requirements will result from further studies of continuous cultures, such as are described in a preliminary way. It should then be possible to maintain continuous cultures at high efficiencies for very long periods of time.

REFERENCES

1. Foster, John F.; and Litchfield, John H.: A Continuous Culture Apparatus for the Microbial Utilization of Hydrogen Produced by Electrolysis of Water in Closed-Cycle Space Systems. *Biotech. and Bioeng.*, vol. VI, 1964, pp. 441-456.
2. Bongers, Leonard H.: Sustaining Life in Space - A New Approach. *Aerospace Med.*, vol. 35, 1964, pp. 139-144.
3. Bongers, Leonard: Chemosynthetic gas exchanger. Second quarterly progress report for period ending December 18, 1963, ER-13270-2, Contract NASw-713, Martin-Marietta Co., Baltimore, 1963, p. 3.
4. Calloway, Doris H.; and Margen, Sheldon: Semiannual report on NASA Grant NGR-05-003-089 for the period 1 June 1965 to 30 Nov. 1965. Dept. Nutr. Sci., Univ. of Calif., Berkeley, 1965.
5. Coulombe, J. J.; and Favreau, L.: A New Simple Semimicro Method for Colorimetric Determination of Urea. *Clin. Chem.*, vol. 9, 1963, pp. 102-108.
6. Technicon AutoAnalyzer Method File N-1b. Urea Nitrogen. Technicon Instr. Corp., Chauncey, New York, 1965.

ALGAL SYSTEMS FOR BIOLOGICAL FOOD SYNTHESIS

By C. H. Ward

Rice University

and

R. L. Miller

USAF School of Aerospace Medicine

During the past 15 years, research effort on photosynthetic regeneration for space life support has cycled from conception in 1952, through a period of intense activity in the years 1957 to 1962 to the present, where only a few laboratories are actively engaged in applied aspects of the problem. At one time it was common to speak of bioregenerative life support only in classical terms of closed ecological systems. Some idealists have stuck to the premise that closed ecological systems with complete material balance are mandatory. However, it is a fair statement of fact that most bioregenerative research (photosynthesis or otherwise) has been directed toward atmosphere regeneration only, that is, provision of oxygen and removal of carbon dioxide. Only limited attention has been given to solubilization and reuse of human waste products and even less attention to the problem of using algae and other microbes for an acceptable, continuous food supply. Concentration of effort on atmosphere regeneration may be warranted since it can easily be shown that about 85 percent of man's requirements can be satisfied by recycling water and exchanging respiratory gases. However, present indications are that a greater degree of closure will be necessary to make regenerative systems competitive with stored systems for long-term space missions.

This paper will briefly summarize available information on factors known to affect the mass culture of algae (primarily chlorellas) for photosynthetic gas exchange, discuss the current state of development, and describe one experimental program to illustrate the immediate future of bioregeneration by photosynthetic processes. The literature on algal photosynthetic gas exchangers was recently reviewed in detail (ref. 1). Krauss (ref. 2) has written an excellent summary on the nutritional qualities of algae.

FACTORS LIMITING ALGAL GROWTH

Control of a variety of growth factors is required for optimization of algal mass cultures for photosynthetic gas exchange (table I). Factors such as temperature, CO₂ concentration, pH, and nutrient supply are easily controlled and maintained at nonlimiting levels. Pressure and oxygen concentration are generally not controlled, but seldom cause difficulties at ambient levels. Light quality and intensity are also subject to control. However,

the transfer of energy in the form of light and the consequences of light saturation severely limit the efficiency of the photosynthetic process and, in turn, the development of compact algal regenerative systems. In fact, optimization of the photosynthetic process has evolved largely into a problem of illumination engineering.

TABLE I. - FACTORS AFFECTING GROWTH OF THERMOTOLERANT CHLORELLA

Variable	Range	Optimum
Light intensity	0-10,000 ft-c	1600 ft-c
Light quality	0.25-0.7 μ	0.4-0.7 μ
Temperature	25 $^{\circ}$ -40 $^{\circ}$ C	39 $^{\circ}$ C
Carbon dioxide	1-40 mm	Not sensitive
Oxygen	1-160 mm	Not sensitive
pH of medium	3-11	6.0-6.8
Minerals		
Macro	10 $^{-2}$ -10 $^{-3}$	Not sensitive
Micro	10 $^{-6}$ -10 $^{-7}$ M	Not sensitive
Pressure	Vapor pressure water-1 atm	Not sensitive

Liberation of oxygen through the process of photosynthesis requires energy in the form of visible light. Light as a source of energy is not only difficult to generate efficiently, but is also difficult to transfer to the plant pigments where useful work is accomplished. Furthermore, illuminated plants are able to convert only a fraction of the energy absorbed into new cell material. Hence, in the overall process of photosynthetic bioregeneration, efficiency is lost during generation, transfer, and absorption of the energy required to drive the process.

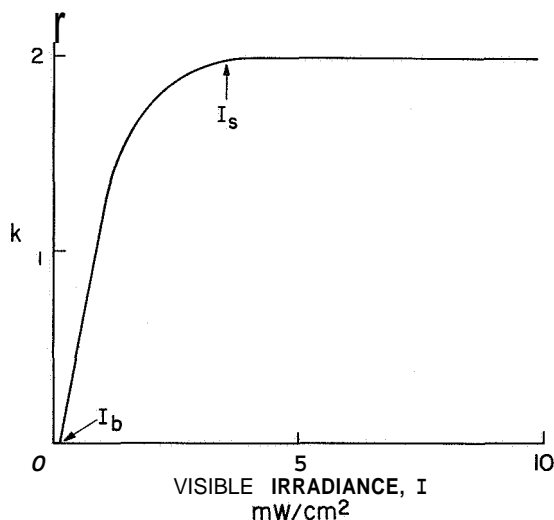


Figure 1.- Specific growth rate, k , as a function of irradiance. I_b denotes the irradiance required to balance respiration. I_s denotes irradiance at which growth rate becomes light-saturated (ref. 1).

Figures 1 and 2 help illustrate the problem of energy transfer to and absorption by algae. Individual algal cells respond to incident light energy in a predictable manner (fig. 1). The ordinate is the cellular growth rate or photosynthetic rate and the abscissa has units of irradiance. The same type of curve would hold for illuminance. It is evident that growth of algae becomes saturated with increasing light intensity. At low intensities, growth rate increases with increasing intensity, and efficiency (given by the slope of the curve) is maximal. The symbol I_b is the compensating intensity or the irradiance required to just

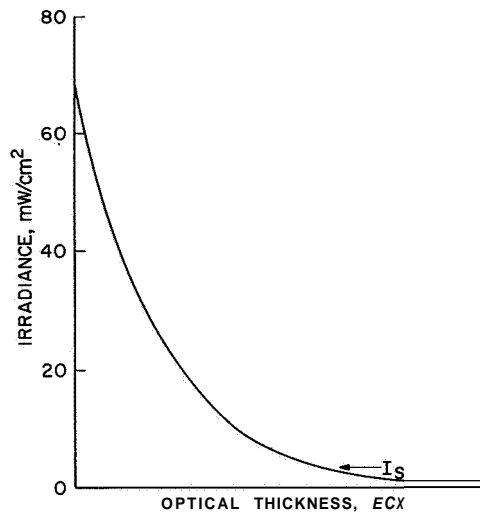


Figure 2.- Irradiance decay in algal cultures as a function of optical thickness (ref. 1).

absorbance curve in figure 2. Starting with some initial irradiance (70 mW/cm^2) at the surface of a panel-type culture vessel, the intensity decays with increasing culture depth x in some approximation to Beer's law. Throughout the culture, gradations in light intensity give rise to gradations in cellular photosynthetic rates which are governed by the response curve in figure 1. The overall rate is a composite of each differential increment of culture thickness and may be determined by integration. Several mathematical treatments have been made on this basis, but no one model has yet accounted for all of the many factors involved.

The model first proposed by Dr. Vannevar Bush in 1952 can be used, within limits, to estimate dense culture performance. The model is based on the assumptions that (1) the culture is exposed to a uniform plane of light in a rectangular (panel-type) culture vessel, (2) all of the light is absorbed by the culture, (3) the light curve can be approximated by two straight lines intersecting at I_s and that I_p is negligible, and (4) light intensity decays exactly according to Beer's law, that is, $I_x = I_0 e^{-cx}$.

If we let

c population density, g/liter

x culture depth

L depth of panel

R production rate, $\text{g/m}^2\text{-day}^{-1}$

k rate of photosynthesis per unit cell mass, g/g-day^{-1} or l/g-day^{-1}

and from assumptions (3) and (4)

maintain the overhead basal metabolism; I_s is the (approximate) light saturating intensity, above which growth rate cannot be increased. Consequently, when irradiance is increased above I_s , efficiency of light utilization falls rapidly.

Efficiency considerations are further complicated in mass culture applications because algae absorb light beyond that which can be productively used. In large-scale culture, maximum productivity and greatest efficiency are obtained with light-limited, optically dense cultures. In optically dense culture, light intensity decays with optical thickness according to the

$$\begin{aligned}
 k &= E_m I & 0 < I < I_s \\
 &= r_m = E_m I_s & I \geq I_s
 \end{aligned}$$

and

$$I = I_x = I_0 e^{-cx}$$

then, by integration, the production rate R becomes

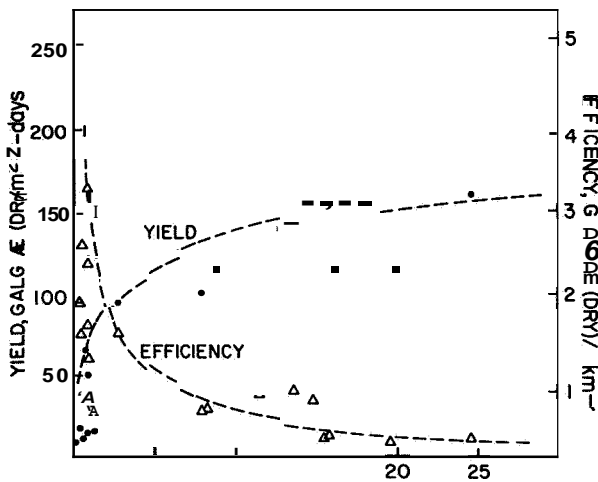
$$R = c \int_0^L r(I_x) dx$$

$$R = c \int_{x_s}^{x_s} r_m dx + c \int_{x_s}^L E_m I_0 e^{-cx} dx$$

$$R = E_m I_s \left(\ln \frac{I_0}{I_s} + 1 \right)$$

and efficiency E becomes

$$E = E_m \frac{I_s}{I_0} \left(1 + \ln \frac{I_0}{I_s} \right)$$



assumed equivalent to irradiance. The data represent the performance characteristics of a number of photosynthetic exchangers reported in the literature and in various governmental and industrial reports. It was not the intent to "fit" the data with the theoretical curves, but only to show the expected relationship between yield on the left ordinate, efficiency on the right, and light intensity (expressed as electrical power input) on the abscissa. Variations in the data are rather striking,

It is of interest to compare the efficiencies shown in figure 3 with the theoretical maximum attainable with artificial light sources. If the maximum (fluorescent lamp) efficiency for the conversion of electrical energy into light energy is 20 percent and the maximum algal efficiency for the conversion of light energy to chemical energy is about 20 percent, the maximum attainable overall efficiency becomes $0.2 \times 0.2 = 0.04$ or 4 percent, which is equivalent to 6.3 g (dry) algae/kWh. The highest efficiencies shown are about half the maximum value.

There are two obvious ways to increase the overall efficiency of photosynthetic regeneration. One is to develop a more efficient light source, and the other is to find a more efficient alga. While prospects for neither are bright, attention is being given to both aspects of the problem. It is now clear that thermotolerant chlorellas, once thought to be faster growing and highly efficient, are no more productive or efficient than low temperature strains when compared in mass culture.

GAS EXCHANGER PERFORMANCE

Numerous algal gas exchangers have been designed, constructed, and at least partially tested. Most are inadequately described, which severely limits interpretation of published data. Typical gas exchanger data for the respiratory support of one man (600 liters O_2 /day) are presented in table 11.

TABLE II.- COMPARISON OF PHOTOSYNTHETIC GAS EXCHANGE SYSTEMS

Author ^a	Light source	Efficiency, percent	One-man requirement		
			Area, m ²	Volume, liter	Power, kW
Wallman et al.	Fluorescent	2.2	9.2	460	7
Ward et al.	Fluorescent	1.5	12.0	100	10
Hannan et al.	Quartzline	.5	1.7	43	48
Matthern and Koch	Quartzline	.2	1.5	46	68
Gafford and Fulton	Sunlight	---	12.0	340	---
Meleshko	Incandescent	---	---	3.5	---

^aor complete citations and additional information, consult Miller and Ward (ref. 1).

No distinction is made between reported values and those calculated from the author's data. Although discrepancies exist in the data, they are more apparent than real. The data suggest an inverse relationship between power and all

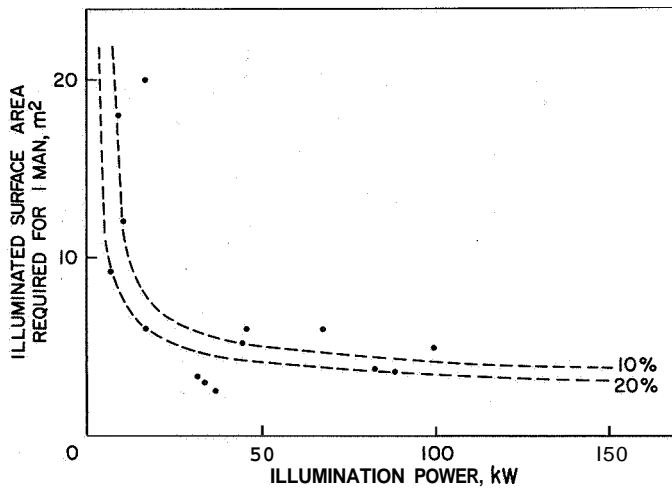


Figure 4.- Illuminated surface area required for one-man support as a function of illumination power (ref. 1).

other parameters listed. The relationship between power and efficiency is illustrated in figure 3. That illuminated surface area and, hence, volume (generally the greater the surface area the greater the volume) are inversely related to power input is demonstrated in figure 4. Data are reported values (ref. 1); the curves represent the Bush equation for lamps of 10- and 20-percent electrical efficiency. A compromise between surface area, volume, and power input is obviously required, depending on whether the design objective is maximum electrical efficiency or minimum gas exchanger mass.

Demonstrated principles of algal metabolism permit calculation of minimum values for a photosynthetic system capable of supporting one man. Present artificial light sources will require approximately 4 kW of power and from 10 to 15 m² of illuminated surface. If continuous direct sunlight at the intensity just outside the earth's atmosphere is used, a minimum of about 6 m² of energy-collecting surface will be required. Both of these alternatives present obvious problems to the design engineer; because of these problems, other methods of bioregeneration are being investigated.

CURRENT DEVELOPMENT

In comparison with the limited information available on alternative biological and physicochemical systems, parameters and limitations governing the development of algal photosynthetic systems are well known. However, existing engineering and performance data do not permit rigorous comparative logistic analyses. The reliability and engineering characteristics of a man-supportive algal system have not been adequately determined. Reported results of human life-support experiments with algal systems are questionable primarily because of the inability to control gas leakage. Thorough engineering analysis and demonstrated reliability are requisite to serious consideration of any biological regenerative system for space application. A current Air Force program is designed to obtain the needed information on algal systems.

The USAF School of Aerospace Medicine has contractually developed a large capacity algal photosynthetic gas exchanger with human life-support capability. The exchanger system has sufficient nominal capacity to provide the CO₂-O₂ exchange necessary for support of two men in a closed environment. Since the exchanger was designed as a research tool and not a flying prototype, no attempt was made to conserve total hardware volume or weight. The primary emphasis in design was to ensure reliable operation, ease of maintenance, and to provide complete instrumentation.

The exchanger system consists of two carts (fig. 5). The instrument-process cart (left) contains the instrumentation and hardware required for control, as well as the gas and liquid pumping, contacting, and separating machinery. The culture cart (right) contains the algae growth panels, lamp banks, and air blowers and dampers necessary for temperature regulation.

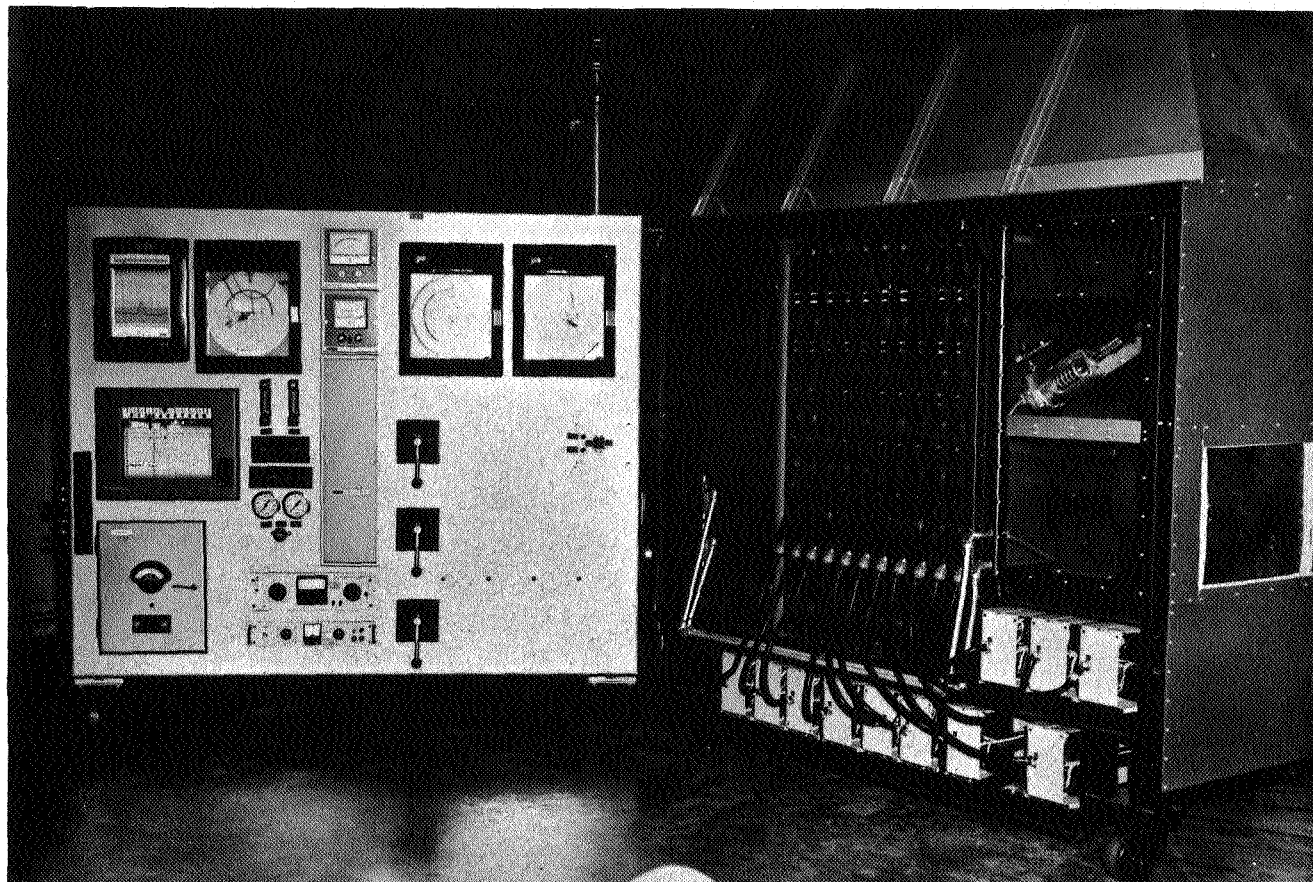


Figure 5. - Front view of two-man capacity algal photosynthetic gas exchanger.

The algal culture is illuminated in thin panels sandwiched between banks of fluorescent lamps (fig. 6). Important design characteristics of the illumination system are given in table 111. Nominal design capacity to support two men requires 10 culture panels and 11 lamp banks. Each culture panel consists of 16 tubes, 110 cm long and 7.5 cm wide, with an illuminated culture depth of 0.5 cm. Hence, the light path is 2.5 mm with bilateral illumination. Each culture panel has 2.65 m² of illuminated surface (both sides) and holds about 7.4 liters of culture including the tubes and turnarounds.

Each lamp bank contains 24 fluorescent lamps (110 W, Sylvania 48T12 CW/VHO) for a total illumination power requirement of 29 kW. This power requirement is a design characteristic and is still subject to testing. Conservative design criteria were employed throughout the exchanger system and it is possible that less power will be required to support two men.

The algal culture is continuously circulated by a centrifugal pump through the gas contactors, gas separator, and illuminated culture panels

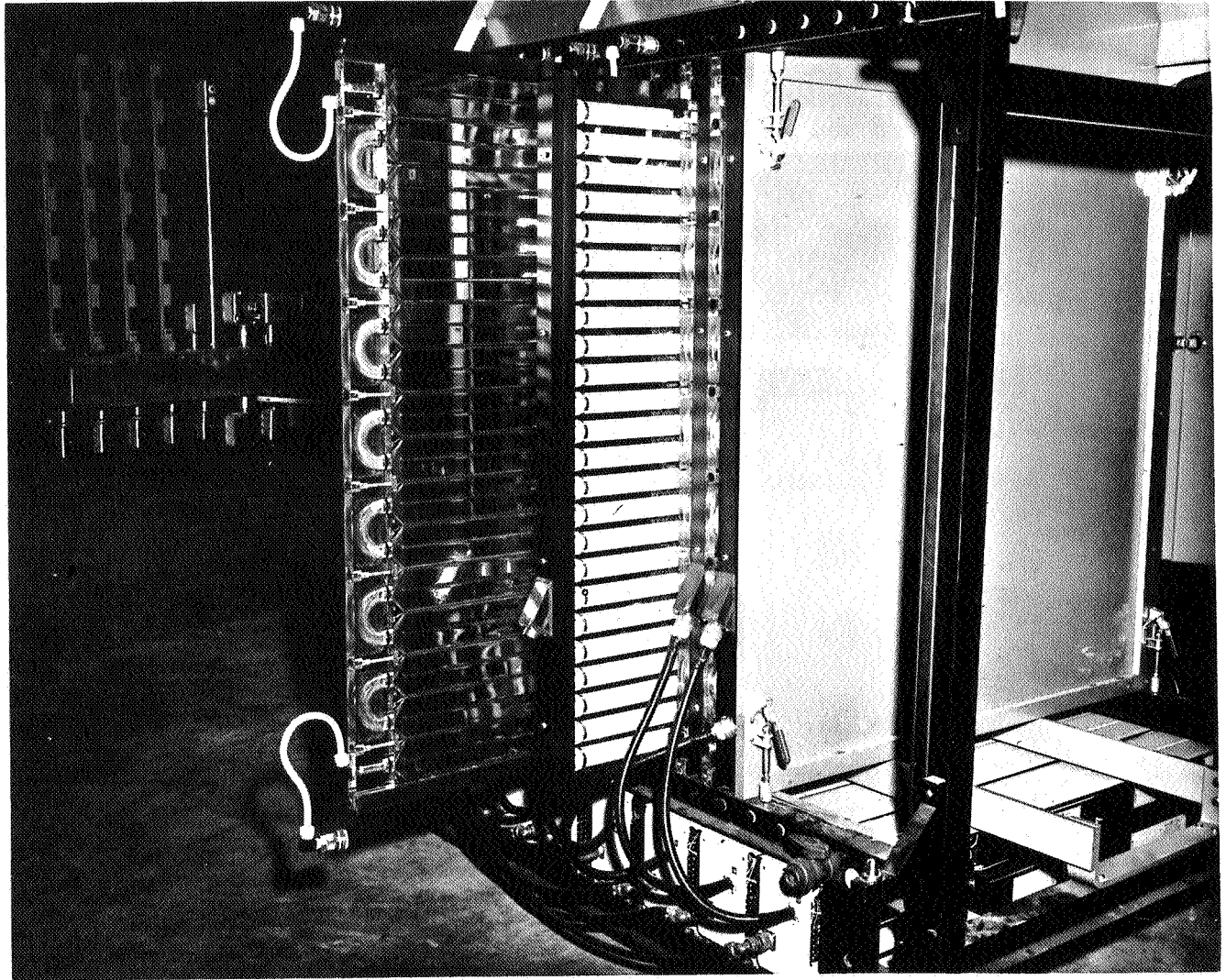


Figure 6. - Culture cart of two-man capacity algal photosynthetic gas exchanger showing culture panel and lamp bank.

TABLE 111.- TWO-MAN PHOTOSYNTHETIC EXCHANGER DESIGN CHARACTERISTICS

Parameter	Quantity
Number of algae panels	10
Number of lamp banks	11
Culture volume	
Total	100 liters
Illuminated	74 liters
Illuminated surface area	26.5 m ²
Illuminated culture depth	5 mm
Illuminated power input	29 kW
All other power input	4 kW

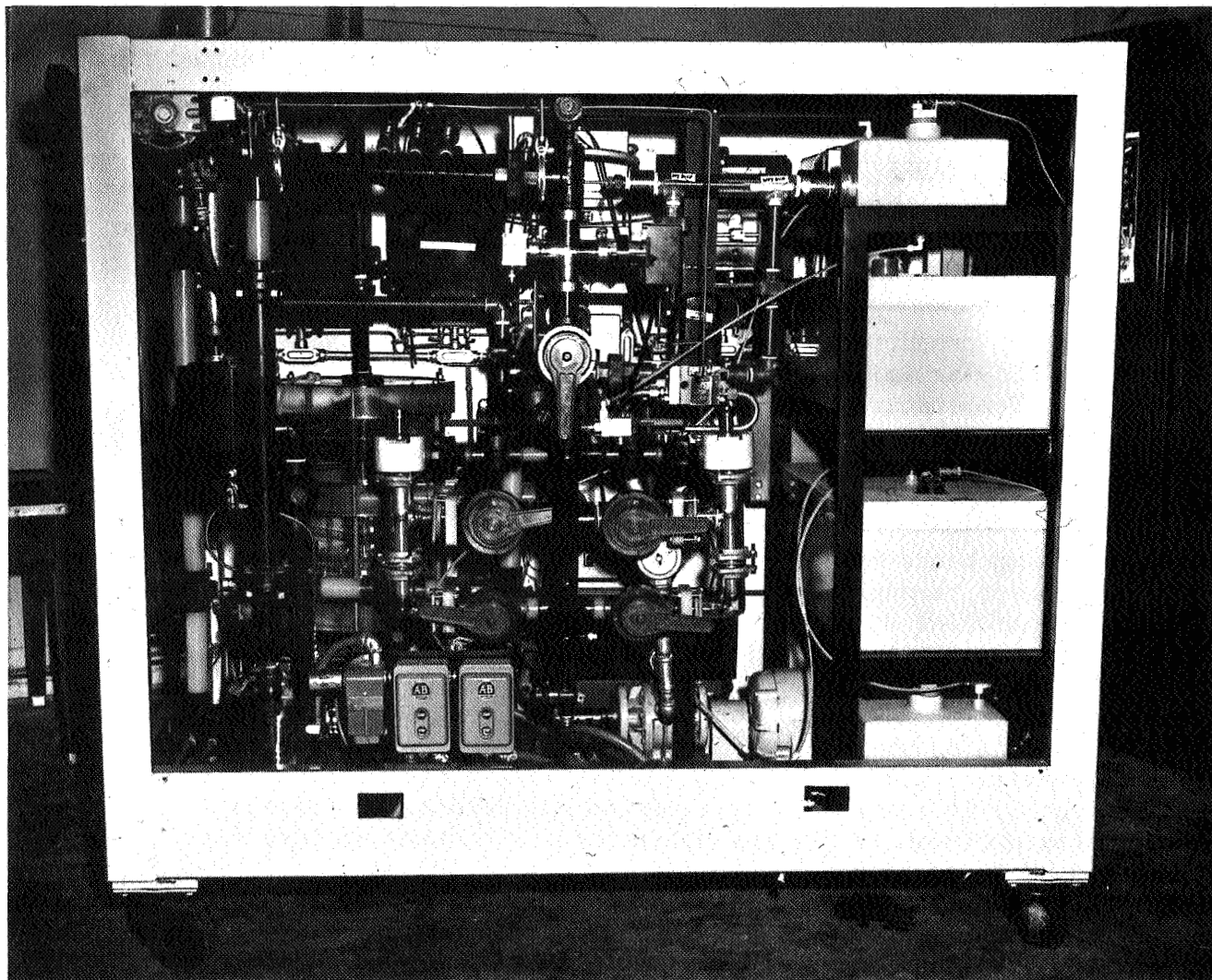


Figure 7.- Instrument-process cart of two-man capacity algal photosynthetic gas exchanger showing pump, plumbing, liquid-gas contactors, centrifugal liquid-gas separator, and other control equipment.

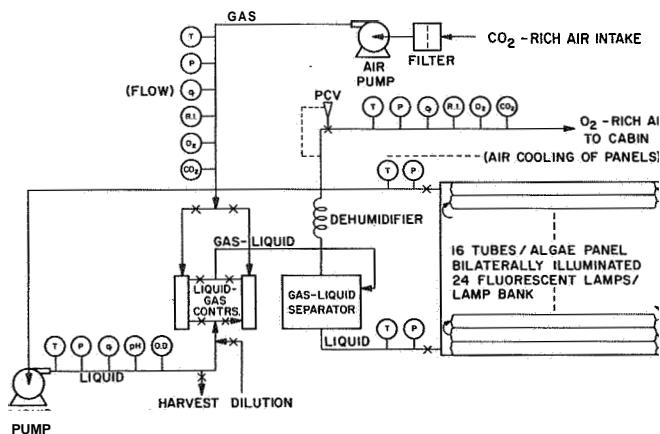


Figure 8.- Flow schematic of two-man capacity algal photosynthetic gas exchanger.

(figs. 7 and 8). Gas-liquid phase contacting is accomplished by sparging CO_2 -rich air under pressure through sintered stainless steel filters in the liquid flow line. Two separate (but identical) gas contactors are operated in parallel so that one may be taken off-line at any time for cleaning and maintenance. The gas is separated by centrifugal action on the two-phase flow stream applied by high velocity tangential entry into a cylindrical vessel. The O_2 -rich gas is vented off the top of the separator, dehumidified, depressurized, analyzed, and finally exhausted to an environmental chamber or to the atmosphere as the experiment dictates.

Projected operational capabilities of the exchanger system are given in table IV. The carbon dioxide oxygen exchange capacity may be varied from 0 to 1200 liters/day depending upon the number of culture panels. Both culture

TABLE IV.- TWO-MAN PHOTOSYNTHETIC EXCHANGER
OPERATIONAL CAPABILITIES

Parameter	Quantity
O ₂ production	0-1200 liters/day
CO ₂ assimilation	0-1000 liters/day
Temperature control range	25° to 55° ±1° C
Pressure control range	0-10 psig
Density control range	0-20 g/liter (dry weight)

panels and lamp banks may be easily added or removed during operation by means of quick-connect hardware. Culture temperature may be maintained to within ±1° C and controlled at any desired point from 25° to 55° C. The wide control range allows considerable flexibility in the choice of algal species from the common mesophilic (25° C) forms to the true thermophilic (52° C) strains. The exchanger system was designed to operate under positive pressure up to 10 psig. This was done to ensure against inboard gas leakage which has seriously compromised previous life support experiments with photosynthetic exchangers. Culture density is controlled by a continuous reading photoelectric colorimeter located upstream to the gas contactors. Constant optical density is maintained by triggering dilution and harvest of the algal culture. External to the exchanger system, facilities are available for separation of the algal cells from the harvested culture. The recovered medium can be pasteurized, replenished with nutrients, and reused in the recycle mode of operation.

The School of Aerospace Medicine photosynthetic exchanger is a research instrument specifically designed for the study of the logistics and reliability of regenerative life support by green plant photosynthesis. To accomplish the desired objectives, an experimental program has been devised that can be divided into four sequential phases.

Phase I is under way and consists of preliminary testing of the various components of the exchanger system. Measurement and control subsystems will be evaluated over the entire range of anticipated use. Necessary minor modifications will be accomplished during this period.

Phase II will be a complete performance test of the gas exchanger to evaluate oxygen production and CO₂ assimilation rates as functions of population density and illumination power input (light intensity). Performance will be optimized for "once through" operation (no recycle of spent medium) and for the mode in which spent medium is replenished with nutrients and recycled back for culture dilution.

Phase III will be a relatively long-term (60 day) test to evaluate the stability and maintainability of the algal culture and exchanger system under

conditions of continuous simulated closed-loop operation. This test will be accomplished with recycle operation and will evaluate performance under both light and CO₂-limited conditions. In actual life support applications, the photosynthetic rate will be limited by light input for purposes of efficiency and by CO₂ as a consequence of the respiratory rate of the human component. Hence, considerable effort will be directed in phase III to defining the inter-relationships in this mode of operation. A similar developmental program on plant nutrient reclamation from human wastes by activated sludge solubilization will proceed concurrently. Waste solubilization will be incorporated into the regenerative process to add an additional degree of closure to the life support system.

Phase IV will culminate in a manned experiment in semiclosed loop operation with photosynthetic gas exchange, waste solubilization, and water recovery. The first experiments will be short-term (1-5 days), employing primarily photosynthetic gas exchange. Longer experiments will follow incorporating nutrient (waste) reclamation and water recovery. The program goal is a long-term (30-60 days) demonstration where all of man's requirements (with the exception of food) are provided in a sealed environment by regenerative processes.

The time schedule for completion of this program is not and cannot be well defined. Phase I is now underway and will likely require from 6 to 12 months for completion. Phases II and III should require a minimum of 6 months each and phase IV may require a year or longer. It is hoped that alternative processes will be similarly evaluated during this period. Then and only then can accurate logistic comparisons be made which include the primary criterion of reliability.

CONCLUDING REMARKS

Photosynthesis as a means of bioregenerative life support remains an active area of research. The justification, if, indeed, one is required at this stage of development, is that there is no proven alternative for either atmospheric regeneration or food production.

Photosynthesis as a regenerative process offers several advantages over other possible biological and physicochemical methods. Photosynthesis requires no environmental extremes of temperature, pressure, or chemical compounds known to be incompatible to man. In addition, the gas exchange ratio obtained through photosynthesis is flexible and can be controlled to match that of the human. Thirdly, while the energetics of photosynthetic exchange with artificial light are not promising, photosynthesis becomes more competitive from an energy standpoint when use of sunlight energy is considered. Hence, it seems highly probable that for certain applications, such as a fixed planetary base, bioregeneration by photosynthesis will be an attractive alternative.

REFERENCES

1. Miller, R. L.; and Ward, C. H.: Algal Bioregenerative Systems. Atmosphere in Space Cabins. K. Kammermeyer, ed., Appleton-Century-Croft Publishing Co., New York, 1966, and USAF Report SAM-TR-66-11, Brooks AFB, Texas.
2. Krauss, R. W.: Mass Culture of Algae for Food and Other Organic Compounds. Am. J. Botany, vol. 49, 1962, pp. 425-435.

APPENDIX

ATTENDEES AT THE CONFERENCE ON THE CLOSED LIFE-SUPPORT SYSTEM

Aerojet-General	Carnation Company Research Laboratory
Dr. R. P. Geckler	C. C. Loo
AiResearch	Douglas Aircraft Company, Inc.
W. L. Burriss	R. Barker
G. Davis	F. E. Littman
W. O'Reilly	EMC Corporation, Santa Clara, Calif.
J. Rousseau	R. Davidson
Archer Daniels Midland Company	R. Griffin
J. Lee	R. A. Lampi
Arde, Incorporated	H. Nosrati
J. D. Zeff	EMC Corporation, Princeton, N. J.
Arequipa Foundation	N. J. Brunsvold
D. A. Salzberg	General American Research Division
Department of the Army	W. Jasionowski
J. E. Malcolm	General Dynamics/Convair
Autonetics	R. Armstrong
S. M. Lee	G. L. Drake
J. J. Licari	W. Johnson
Battelle Memorial Institute	C. D. King
J. E. Clifford	L. L. Short
R. Filbert	E. A. Zuraw
Dr. J. F. Foster	General Electric Company
Boeing Company	L. Cooper
R. K. Olsen	T. K. Slaweki
D. Taylor	Grumman Aircraft Engineering Corp.
California Institute of Technology	A. Bruno
Dr. J. E. McKee	Hamilton Standard
H. G. Schwartz, Jr.	B. Lockwood, Los Altos, Calif.
	Dr. E. G. Vail, Windsor Locks
	Hazelton Laboratories, Inc.
	Dr. G. V. Levin

IIT Research Institute

Dr. E. S. Freeman
C. K. Hersh
Dr. G. Sumyk

LTV, Astronautics Division

W. B. Cassidy

Litton Space Sciences Laboratories

W. Elkins
R. Rocco

Lockheed Missiles and Space Co.

J. E. Acker
T. E. Adamson
Dr. G. A. Albright
Dr. E. C. Ammann
G. M. Freedman
Dr. W. M. Helvey
Dr. C. J. Hoffman
R. J. Jaffe
R. B. Jagow
Dr. A. E. Levy-Pascal
J. H. Osiecki
Dr. L. L. Reed
Dr. A. J. Robell
J. M. Smith
R. S. Thomas

Marquardt Corporation

C. E. Hansen
D. B. Krenzel

Melpar, Incorporated

J. H. Chaudet

Midwest Research Institute

W. J. Dunden
J. R. Perrin

MSA Research Corporation

J. W. Mausteller
C. H. Staub

National Academy of Sciences

Dr. F. G. Favorite

NASA Headquarters

A. L. Ingelfinger

NASA, Langley Research Center

D. C. Popma

Naval Air Engineering Center
Philadelphia, Pennsylvania

E. J. Boscola

New Mexico State University

J. E. Weiss

North American Aviation

G. N. Hoover
E. L. Korb
D. Watanabe

Northrop Space Laboratories

Dr. D. L. Dyer
E. R. Glazier

Republic Aviation

J. Colovin
D. MacDonald
Mrs. P. Riely

Research Institute for Advanced
Studies

Dr. L. Bongers

Rice University

Dr. C. Ward

Schwarz BioResearch Incorporated

D. R. Schwarz

Stanford Research Institute

Dr. M. Goldman
M. H. Kuo

Thiokol Chemical Corporation

Dr. M. Cohen
Dr. H. Burwasser

TRW Systems

A. P. Shlosinger
Dr. H. P. Silverman

U. S. Army Natick Laboratories

R. O. Matthern

USAF School of Aerospace Medicine
Brooks AFB, Texas

Dr. R. L. Miller
Dr. S. S. Wilks

University of California, Berkeley

Dr. D. Calloway
D. Goldbloom
R. Pertel
C. Waslien
Dr. C. G. Golueke

University of Miami

Dr. S. W. Fox
Dr. G. T. Lewis

Whirlpool Corporation

R. Grace

Wisconsin Alumni Research Foundation

P. O. Nees

Whittaker Corporation

C. Fredricks
C. Hill

"The aeronautical and space activities of the United States shall be conducted so as to contribute . . . to the expansion of human knowledge of phenomena in the atmosphere and space. The Administration shall provide for the widest practicable and appropriate dissemination of information concerning its activities and the results thereof."

—NATIONAL AERONAUTICS AND SPACE ACT OF 1958

NASA SCIENTIFIC AND TECHNICAL PUBLICATIONS

TECHNICAL REPORTS: Scientific and technical information considered important, complete, and a lasting contribution to existing knowledge.

TECHNICAL NOTES: Information less broad in scope but nevertheless of importance as a contribution to existing knowledge.

TECHNICAL MEMORANDUMS: Information receiving limited distribution because of preliminary data, security classification, or other reasons.

CONTRACTOR REPORTS: Scientific and technical information generated under a NASA contract or grant and considered an important contribution to existing knowledge.

TECHNICAL TRANSLATIONS: Information published in a foreign language considered to merit NASA distribution in English.

SPECIAL PUBLICATIONS: Information derived from or of value to NASA activities. Publications include conference proceedings, monographs, data compilations, handbooks, sourcebooks, and special bibliographies.

TECHNOLOGY UTILIZATION PUBLICATIONS: Information on technology used by NASA that may be of particular interest in commercial and other non-aerospace applications. Publications include Tech Briefs, Technology Utilization Reports and Notes, and Technology Surveys.

Details on the availability of these publications may be obtained from:

**SCIENTIFIC AND TECHNICAL INFORMATION DIVISION
NATIONAL AERONAUTICS AND SPACE ADMINISTRATION**

Washington, D.C. 20546