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AMXFC REPORT NO. 1-63

DEVELOPING AN UNCONVENTIONAL FOOD, ALGAE, BY CONTINUOUS CULTURE UNDER HIGH LIGHT INTENSITY

Interim Report

February 1963

ARMED FORCES FOOD AND CONTAINER INSTITUTE
U.S. ARMY QUARTERMASTER RESEARCH AND ENGINEERING CENTER
CHICAGO 9, ILLINOIS

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PROJECT: Unconventional means of food production

DEVELOPING AN UNCONVENTIONAL FOOD, ALGAE, BY CONTINUOUS CULTURE UNDER HIGH LIGHT INTENSITY

Interim Report

by

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DEVELOPING AN UNCONVENTIONAL FOOD, ALGAE, BY CONTINUOUS CULTURE UNDER HIGH LIGHT INTENSITY

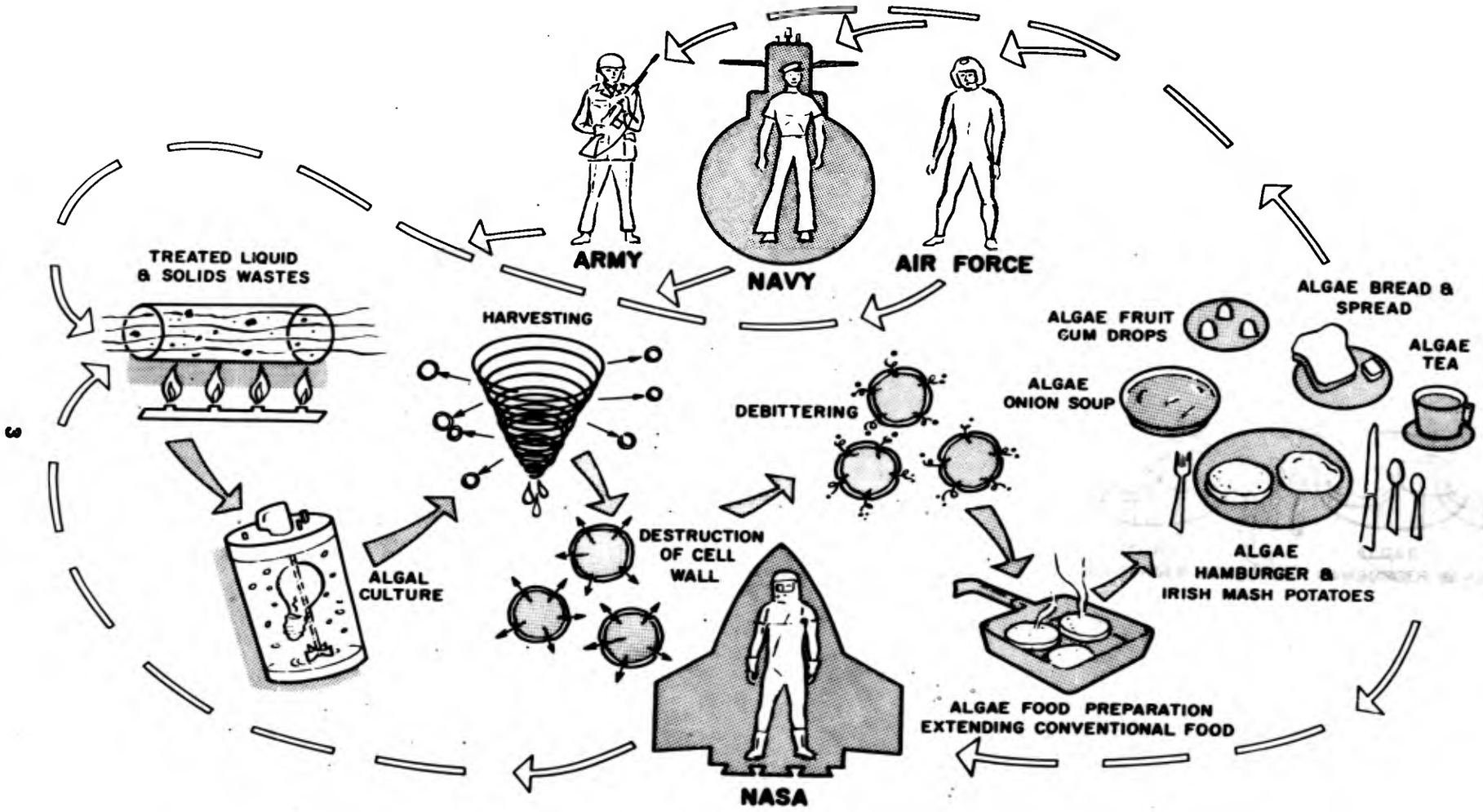
Introduction

Both farm and factory are required in the production of conventional foods. To produce unconventional foods, like algae, would require only a factory since growing and manufacturing would be done under the same roof, skipping the agricultural step with all of its problems and heavy labor. To the U. S. Army, which has to provide foods for every conceivable military situation, unconventional foods are of great interest. A successful, continuous, high-volume operation could be used in feeding indigenous populations of other nations in a siege situation, or to supplement the food supply of our own nation in the event of atomic or biological warfare with its inevitable result -- widespread damage to conventional food production and storage facilities. Closed ecological systems based on unconventional foods will be applicable to other situations -- two quick examples are military operations in non-agricultural areas such as the Arctic and Antarctic and non-military uses such as space exploration.

At the Armed Forces Food and Container Institute, the emphasis of the algae studies has been placed on the production and development of algae as a food. Several progressive steps are required to convert waste products into palatable nutritious food. If, as in a closed ecological system, human waste products are to serve as the source of nutrients for the culture of algae, sterilization by heat or radiation of the wastes will be required. The algae will then be cultured

photosynthetically. It has been successfully demonstrated that human urine can be used as the sole nitrogen source in a medium for algae grown in a high intensity light steady state continuous culture system. Presently, the algae is harvested by differential centrifugation. Work is progressing on the destruction of the cellulose cell wall so as to free the nutritious protoplasm held within the cell. Of the several methods developed by the Armed Forces Food and Container Institute for the removal of the bitter principle from algae, methanol extraction, ethanol extraction, and the preparation of a roux have proved most successful. The debittered algae has been used as an extender of conventional foods. A complete meal could be prepared from some of the algae recipes developed. Such a meal would include an onion soup appetizer, mashed potatoes, hamburgers, an algae spread for use on an algae bread with fruit bars as a dessert and algae tea as a beverage. In Figure 1, the pathway for conversion of waste products to nutritious palatable food is depicted. The NASA civilian in space, the sailor aboard a submarine, the G. I. or Air Force Serviceman in isolated Arctic situation may all find use for this system.

In early stages of the algae work, one of the authors became interested in developing a small compact algae culture system. Following a suggestion to use a completely mixed continuous system for the culture of algae, made by Burk of the National Institute of Health, Koch began work on such a system. The culture system to be described is based on the general concepts of Burk and Koch.



CONVERSION OF WASTES TO PALATABLE FOOD

FIG. 1

Description of Units Comprising System

A schematic diagram of the culture chamber is shown in Figure 2, a schematic of the cooling system in Figure 3, and a diagram of the complete photosynthetic algal culture system in Figure 4.

Chamber

The chamber was made of an 11-inch piece of five-inch diameter acrylic plastic tube having a wall thickness of 1/4-inch. The top and bottom flanges were made of one-inch plastic.

Mixer

Mixing was accomplished using a General Electric synchronous motor, 1/20 H.P., 1750 RPM, 115V, 1.6 A, which drove a 1/4-inch stainless steel shaft containing two 4-blade 1-1/4-inch diameter by 1/4-inch stainless turbine impellers. A superior Electric Type 116 powerstat was used to control the speed of mixing.

Light and Cooling

Four General Electric 1500 T3Q/CL-277V, 1500 watt tungsten filament lamps were used for illumination.

A superior Electric Powerstat Model 1156C-2S, 0-280 watt, 45 amp was used to regulate the voltage to the lamps. When operating at full voltage (277 volts) each of these lamps give off 33,000 lumens which is approximately three times the illumination of sunlight as measured on the surface of the earth. Unfortunately, light in the infrared spectrum comprises 85 to 87 percent of the total energy output from the lamps and had to be absorbed as heat by distilled water in a cooling jacket around the lamp. This jacket was made of an inner 19 mm

Pyrex glass tube and an outer 25 mm tube between which 4°C. distilled water was circulated. This comprised the primary cooling system.

The cooling water to the primary cooling system was pumped from a heat exchanger using an Eastman E-7 pump. The flow rate was controlled by a Superior Electric Type 116 powerstat. A powerstat was used rather than a throttling valve in order to reduce wear on the motor brushes. All the connecting piping was 5/16-inch stainless steel. The secondary cooling system consisted of a coil of stainless steel tubing through which cooling water was pumped from the heat exchanger by an Eastman E-7 pump, the flow rate again was regulated by a powerstat. The discharge gas from the unit was cooled by water from the pump serving the secondary cooling system. A portion of the absorbed moisture was condensed and returned to the culture through the foam breaker. Two Honeywell Versa-Tran transistorized amplifier relays Model R7081A-C with thermistor L7038-C probes were used for temperature control. One was used to actuate a ¼ inch Skinner stainless steel solenoid valve (No. V5D-9900S) in the secondary cooling system and the other was used as a control to the light circuit to prevent the temperature of the culture from exceeding a specific value.

The heat exchanger was made of a double coil of 3/8-inch copper tubing, painted with a bitumastic paint, which served as the refrigeration coils connected to a 5 H.P. Coplamatic condensing water cooled unit Model W5000. The distilled cooling water was continuously stirred by a Lightning Model XP mixer. The gross volume of the polyethylene tank serving as the heat exchanger was 30 liters.

A Weston stainless steel dial thermometer was used to measure the average temperature of the culture.

Nutrient [redacted]

The nutrient was pumped from a 10-liter reservoir by means of a New Brunswick peristaltic pump, Model PA 53, through 1/8-inch surgical tubing to a capillary tube which discharged at mid-depth in the culture. The peristaltic pump was actuated by a Delmar Scientific Laboratory four-minute timer.

Harvest

The harvest system was merely an overflow device made from a 5/16-inch stainless steel tube bend which could be set at any desired level. An open end tygon tube carried the harvest into a 10-liter carboy reservoir.

Gas System

The carbon dioxide and air were filtered, dried, metered, and mixed prior to distribution to the various experimental cultures in the laboratory. The flow to the culture chamber was metered using a Pyrex glass flowmeter which is calibrated with a Precision wet test meter and by positive displacement. The orifice inlet was directly under the eye of the turbine blade mixer. The gas was carried from the top through a 3/8-inch orifice through a short bend of tygon tubing into the side of an 1-1/2-inch diameter plastic tube which served as a foam breaker.

Foam Breaker

In the foam breaker good separation of gas and foam were attained by means of a vortex formed in the liquid culture medium. The vortex was formed using a Labline 1250 magnetic stirrer. The liquid was returned, actually moved back and forth, through a side outlet located at the bottom of the foam breaker which in turn was connected to the culture chamber 1-3/4 inches below the surface of the liquid.

Operation

Capacity of Culture Chamber

The total liquid capacity of the culture chamber and foam breaker was 2.85 liters. However, a space of 150 ml was required for gas hold-up and a gas collecting dome. This left a net culture volume of 2.7 liters.

Mixing

Effective mixing served several necessary functions; gas exchange, dissipation of heat, development of effective on-off time for photosynthesis, intimate contact between nutrients and the cells, and uniform distribution of cells and nutrients throughout the culture chamber.

The impeller-design and location and the speed of mixing were chosen based on the experience of Rich (1), Rushton (2,3), Oldshue (4), and Gaden (5). Although the speed of mixing was chosen more or less arbitrarily, the effect of small changes in RPM was not found to affect production. The four lamps which supplied light served as baffles thereby eliminating formation of a vortex.

Light

The maximum light intensity at which the system could be operated was 13,000 lumens per lamp attained at 210 volts. This maximum intensity appeared to be limited by the capacity of the primary cooling system to dissipate the heat of the lamps.

Cooling System

The temperature of culture was maintained at 38°C. ± .5°C. The maximum light intensity which could be used without cooking the algae by the infrared light rays was found to be limited by a gradual increase of 1°C. over a period of no less than 20 seconds. The primary cooling system was operated continuously. When the temperature rose to 38.5°C., a solenoid valve was opened allowing 4°C. water to circulate through the secondary cooling system (5/16-inch stainless steel tubing coils). The secondary cooling system cooled the culture medium to 37.5°C. in five seconds. The solenoid valve was actuated by one of the Honeywell Versa-Tran relays with its thermistor probe. When the flow of cooling water was not being circulated through the secondary cooling system, it was pumped through the outlet gas cooling condenser.

Nutrient Feed

The nutrient feed was regulated by adjustment of the pressure on the tubing in the peristaltic pump and by adjustment of the timer which actuated the feed pump. Nutrients were fed at least once for an interval of six seconds in every four minutes.

The feed rates were obtained by measuring the harvested volume corrected for moisture lost through the gas and quantities of samples taken for analysis. A 40 ml sample was taken for pH, packed cell volume (PCV), and dry weight determinations.

Gas

A mixture of 10 percent carbon dioxide and 90 percent air (by weight) was used throughout the experiment. The gas flow was held constant at 30 liters per hour.

Nutrient Formula

A problem in regard to the media to be used was encountered in growing algae under high intensity lighting (52,000 lumens). When media that supported growth under low intensity lighting (700 lumens) were used, the growth response was not satisfactory. A comparison of growth on various media led to the finding that as the concentration of magnesium and phosphorus was increased, the growth of algae improved. A study of the media suggested by Burk (6) to Electric Boat and that suggested by Meyers to Arthur D. Little, Inc. (7) indicated that higher concentrations of magnesium and phosphorus could be used. Using the same proportions of macro-nutrients as suggested by Burk (6), it was found that algal concentrations up to 8.5 gms. per liter (dry weight basis) could be attained in batch.

With further study and experimentation, a new approach to the development of nutrient formulas was proposed. Krause (8) had reported in 1953 that algae could use 100 percent of the available nitrogen from a medium but never 100 percent of the magnesium or phosphorus. Based on this finding it was assumed that a certain

minimum concentration of nutrients was required by algae to develop a concentration gradient for rapid transport of nutrient across the cell wall and/or for cellular metabolism. What the precise concentrations were was not as important as finding out the maximum production attainable under our system. To determine this maximum, we assumed first that the T-4 medium contained sufficient nutrients for 8.5 gm. growth plus the basic minimum concentrations. If additional growth were required it was postulated that only those nutrients consumed in producing cellular tissue needed to be supplied. An analysis was made of the Chlorella 71105 grown on T-4 medium under high light intensity, with results as given in Table 1. Sufficient macro-nutrients were therefor added to the T-4 medium to support an additional 11 gm. dry weight of algae. This new formula has been designated T-6. Media T-4 and T-6 are given in Tables 2 and 3, respectively.

Figure 5 presents graphically the concept on which criteria for the design of the medium was based. Assuming for simplicity of explanation, that growth vs. concentrations of nutrients is a straight line function, it can be deduced that the slope of the line represents grams of nutrient required per gram of cell tissue; the ordinate intercept therefore indicates the minimum concentration of nutrients required.

Measurements

Only four measurements were required to control the system: pH, packed cell volumes (PCV), dry weights and quantity of harvest. A Beckman Zeromatic pH meter was used for pH determination. Packed cell volumes were run in duplicate using 10 ml. tubes of the type usually used for Wasserman tests. These tubes were centrifuged for 20 minutes

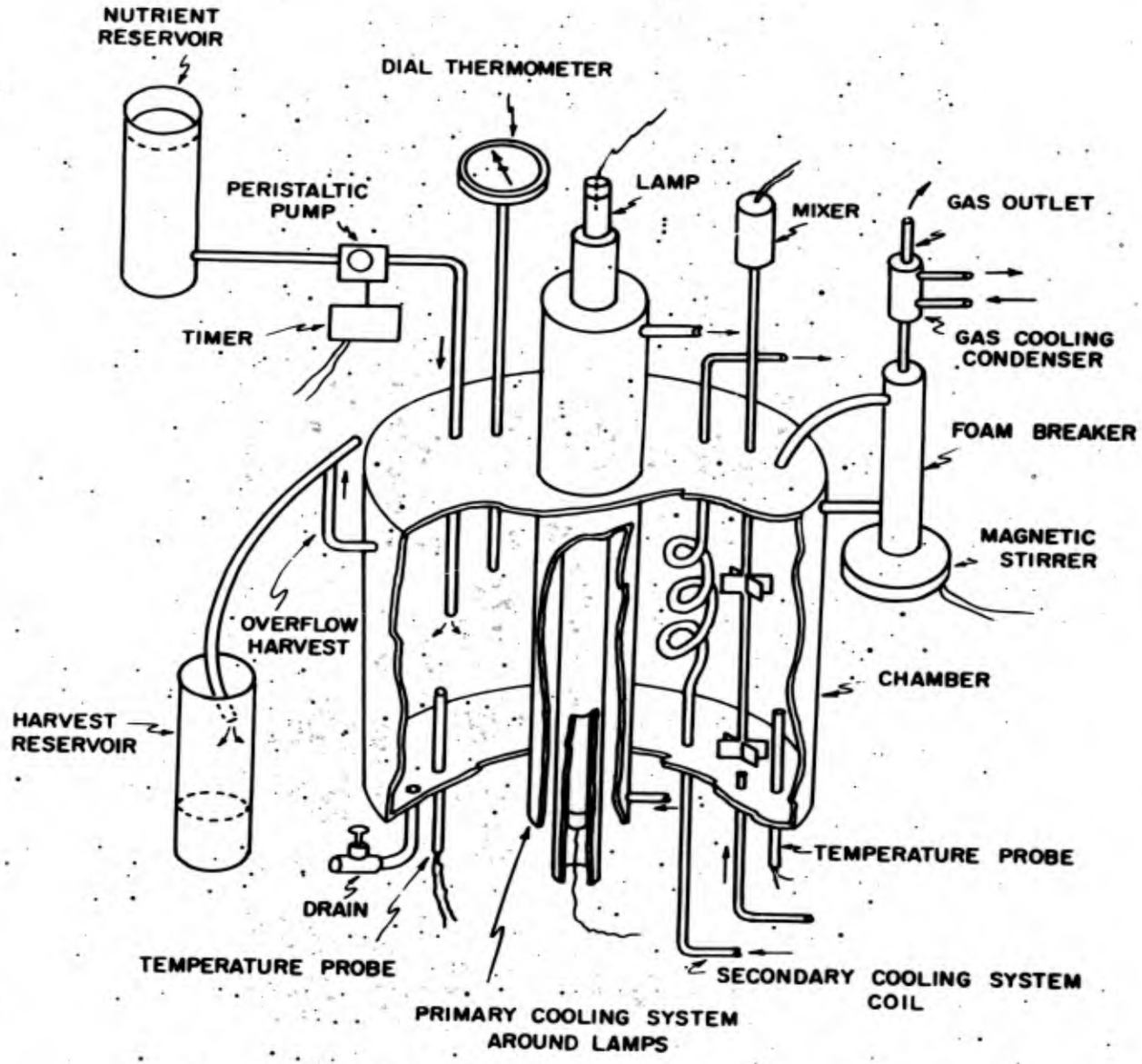


FIGURE 2 SCHEMATIC DIAGRAM OF CULTURE CHAMBER

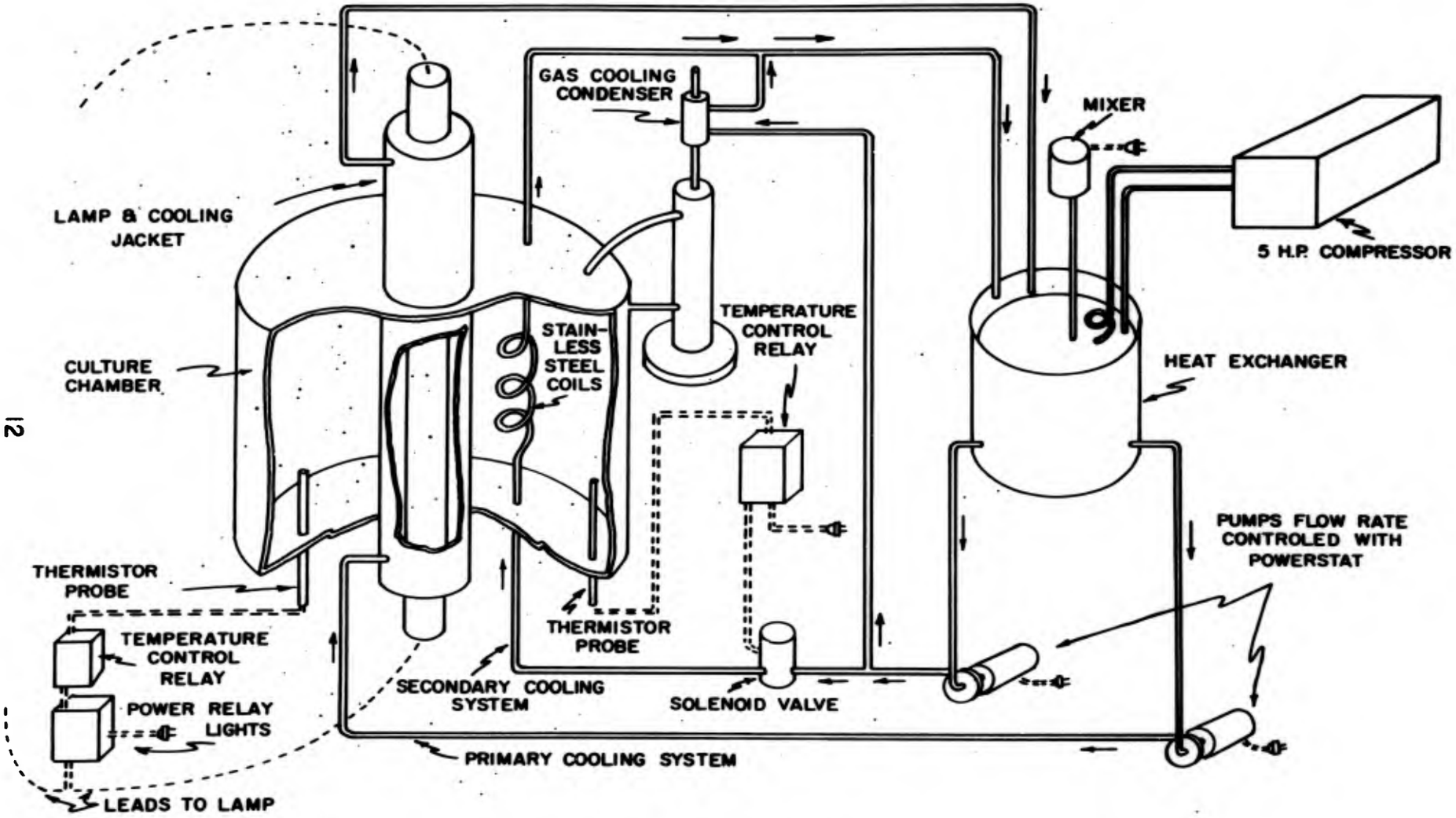
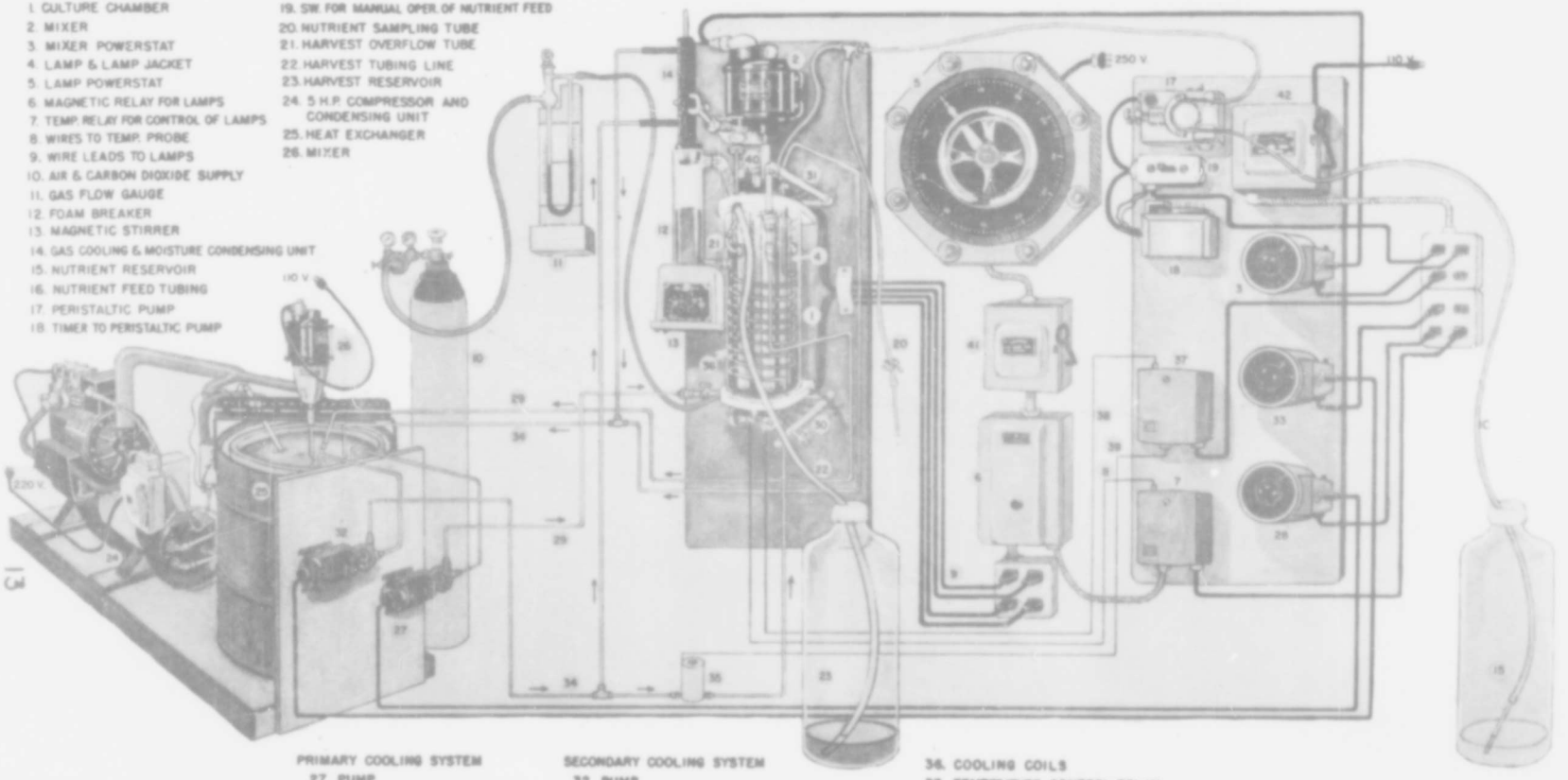


FIGURE 3 SCHEMATIC OF COOLING

FIGURE 4

CONTINUOUS PHOTOSYNTHETIC ALGAL CULTURE SYSTEM



PRIMARY COOLING SYSTEM
- 27. PUMP
- 28. PUMP POWERSTAT
- 29. STAINLESS STEEL PIPING
- 30. DISTRIBUTION MANIFOLD
- 31. COLLECTION MANIFOLD
- 4. LAMP JACKET & LAMP

SECONDARY COOLING SYSTEM
- 32. PUMP
- 33. POWERSTAT
- 34. STAINLESS STEEL TUBING
- 14. GAS COOLING AND MOISTURE CONDENSER
- 35. SOLENOID VALVE CONTROLLING FLOW TO COOLING COILS

- 36. COOLING COILS
- 37. TEMPERATURE CONTROL RELAY FOR SOLENOID VALVE
- 38. WIRES TO TEMPERATURE PROBE
- 39. WIRE TO SOLENOID VALVE
- 40. DIAL THERMOMETER
- 41. MAIN SW. TO LAMPS
- 42. MAIN SW. TO CONTROLS

LAYOUT AND DRAWING BY W.E. BARCZYKOWSKI 19 OCT. 1962

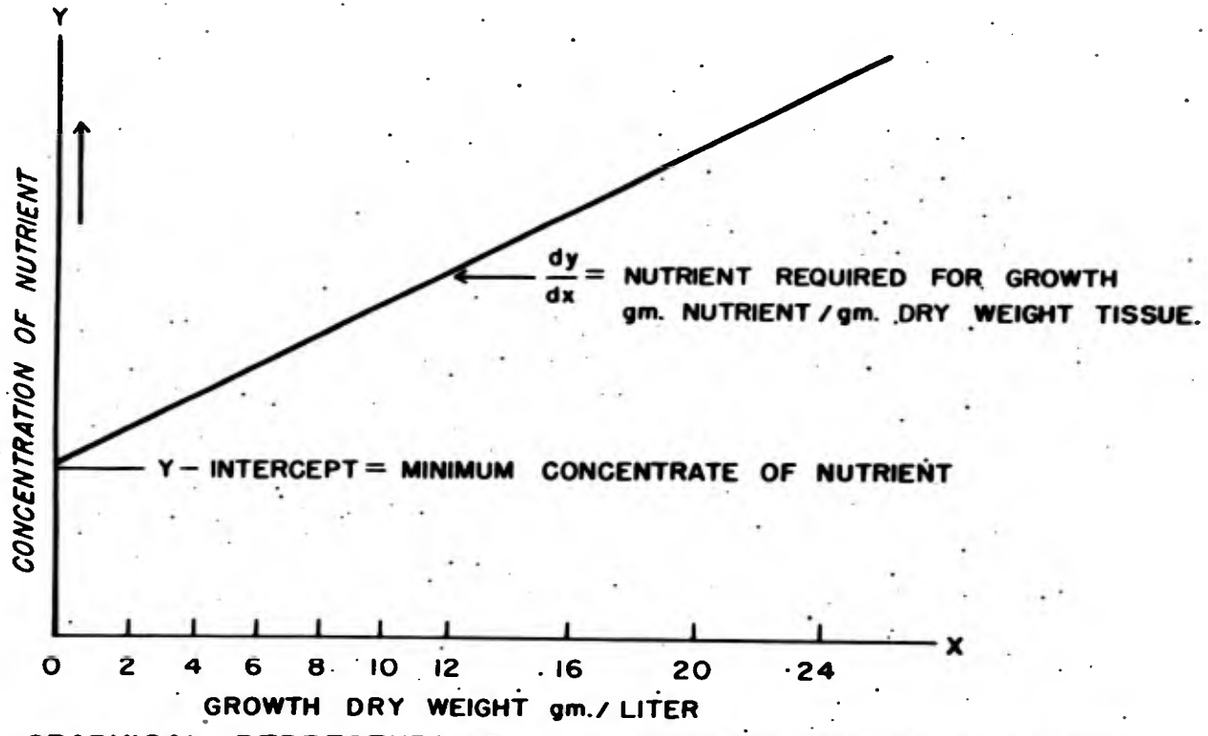


FIGURE 5 GRAPHICAL REPRESENTATION OF NUTRIENT FORMULA DESIGN

at 2660 rpm in a Clay-Adams centrifuge. The duplicate dry weights of algae were made on the packed cells from which the nutrient solution was discarded. The cells were suspended and dried in a Moisture Teller for 38 minutes (by which time a constant weight was attained) at 250°F. The mean moisture content of 27 samples of the Chlorella was determined to be 70 percent.

The feed rates were obtained by measuring the harvested volume corrected for moisture lost through the gas and quantities of samples taken for analysis. A 40 ml sample was taken for pH, packed cell volume (PCV), and dry weight determination.

Production of Algae

The culture chamber was started with approximately 10 gm. dry weight of Chlorella 71105 suspended in T-4 medium. The pH of the medium was adjusted to 6.0. The algae harvested from a batch culture was then suspended in 2.7 liters of medium with the aid of a Waring Blendor. The T-6 medium with a pH of 5.7 was used as feed nutrient. The feed rate was held constant until the cell concentration as measured by PCV remained relatively constant for a minimum period of 47 hours. The feed rate was then changed and the cycle repeated. Table 4 presents the pertinent data.

The pH of the culture varied between 6.3 and 7.2 usually holding in a range of 6.4 to 6.8. When the pH of the culture medium rose above 6.5 for light algal concentrations (4 gm/l) and 7.5 for dense concentrations (7.0 gm/l or more), precipitation of the nutrient salts occurred. A study of the data indicated that no correlation existed

between pH and either growth or feed rate.

Figure 6 is a graph of the effect of feed rate in ml/hr upon the dry weight concentration of algae in the culture. High concentrations at low feed rates cannot be interpreted as producing maximum yields. If the daily yields are computed, using the feed rates and their corresponding algal concentrations as:

$$\text{dry wt in gm/day} = \frac{(\text{dry wt in gm/l})(\text{feed rate in ml/hr})(24\ \text{hr/day})}{1000\ \text{ml/l}}$$

the values of the daily yield column of Table 4 are obtained. These are the values plotted in Figure 7. It may be inferred from the graph that the maximum daily production occurs at feed rates ranging between 150 and 190 ml/hr. At a feed rate of 170 ml/hr, 36 gm/day were attained.

The longest continuous operation of the system was 76 days; during this period only short periods of shutdown were required (for replacement of burned-out lamps and cleaning). On the 76th day, however, a bearing on the mixing shaft failed, requiring a major shutdown for repairs. The culture was kept under refrigeration during the shutdown, and used again when the mechanical failure was repaired and the system again was in operable condition.

Discussion

The T-6 medium was designed to support approximately 19.5 gm/l of algae. The maximum concentration of algae attained was 23.78 gm/l for a feed rate of 26.6 ml/hr. This concentration was a single measurement for which equilibrium was not established in the system. For the same feed rate the concentrations increased from an initial 19.38 to 23.78 gm/l, and then decreased to 20.00 gm/l over a five-day

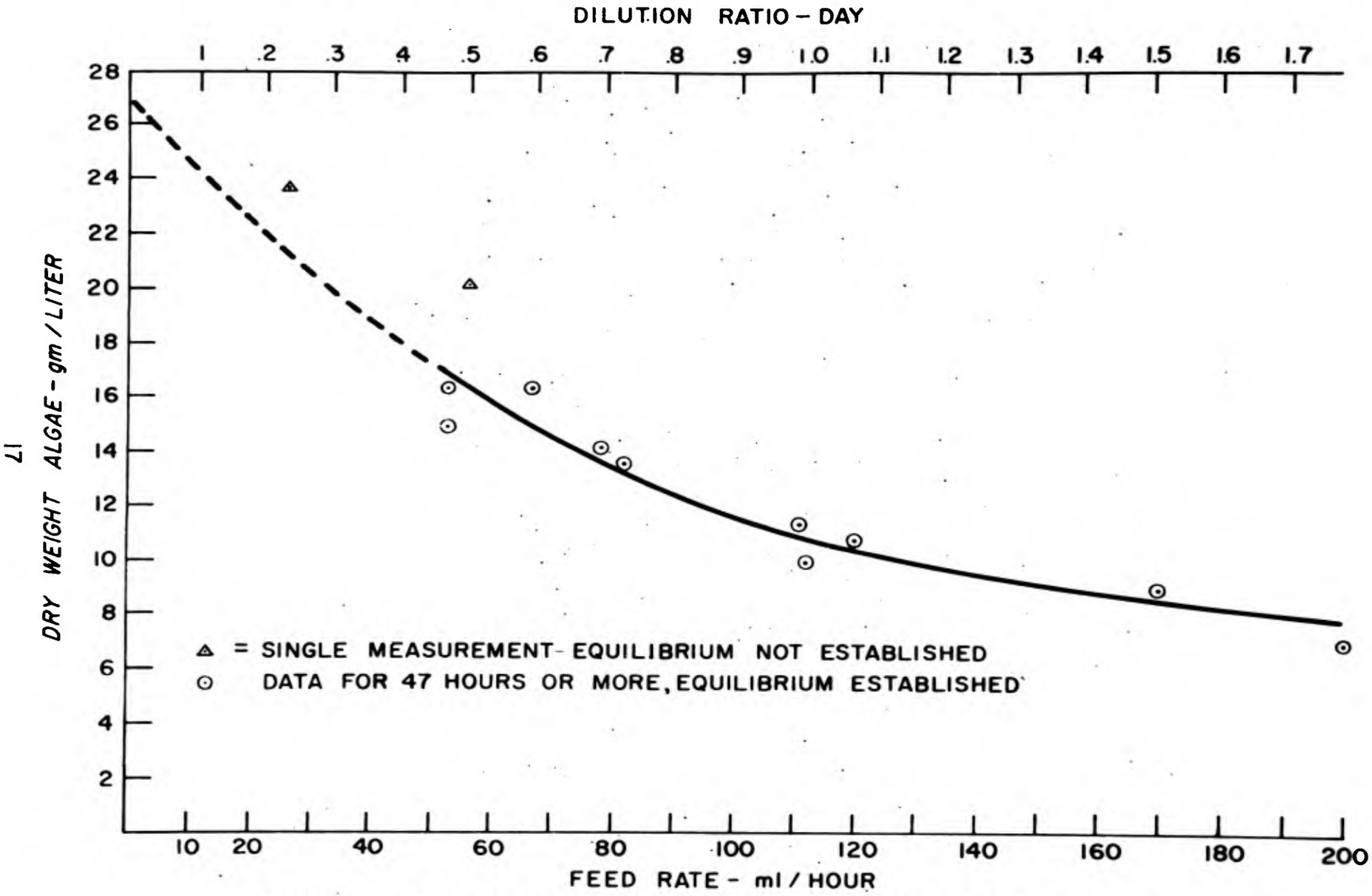


FIGURE 6 EFFECT OF FEED RATE UPON CONCENTRATION OF ALGAE IN CULTURE

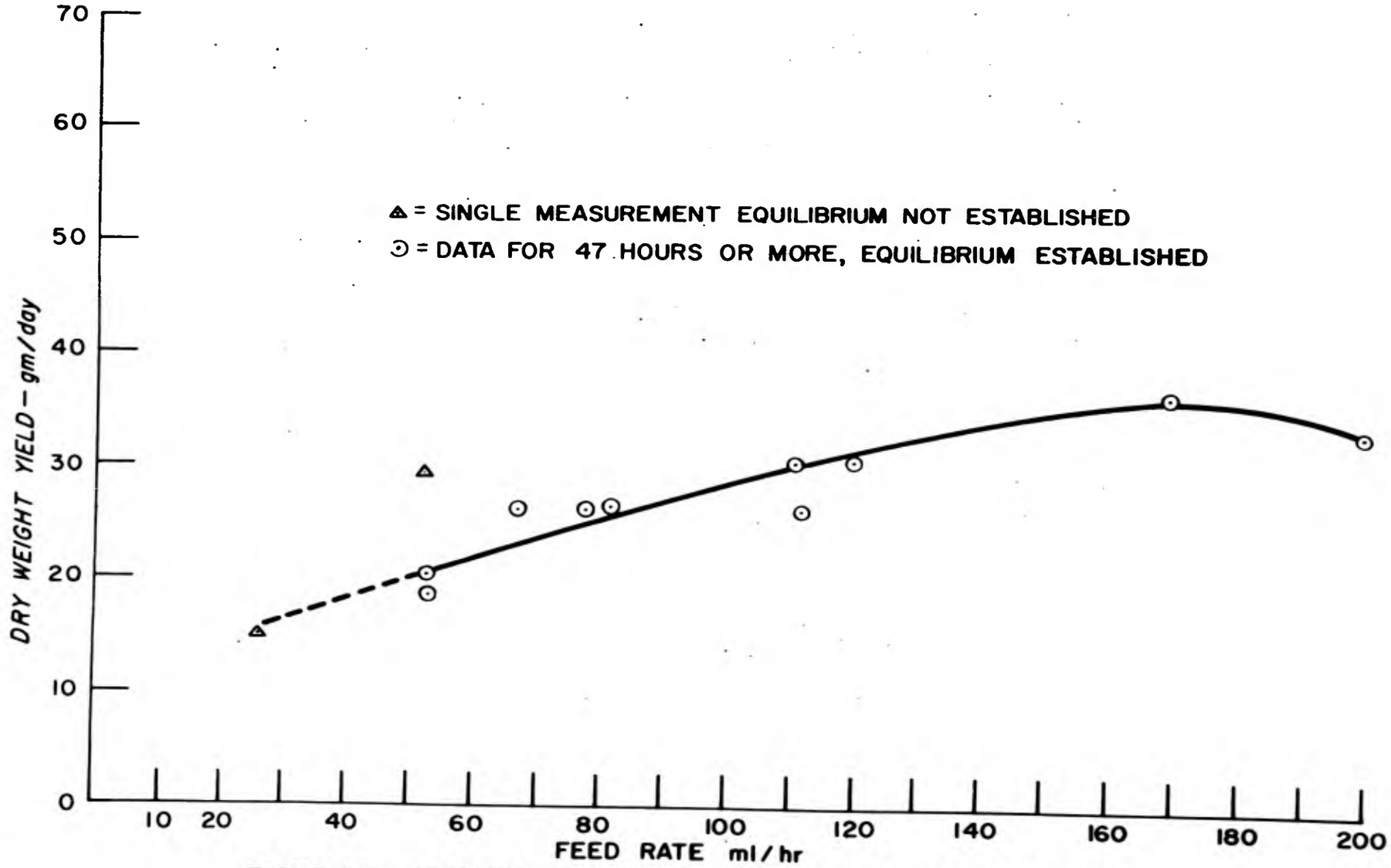


FIGURE 7 EFFECT OF FEED RATE UPON DAILY YIELD

period. The same relative conditions existed for the 21.08 gm/l concentration. As the T-6 medium supported algae concentrations in the neighborhood of 21 gm/l, the results are in good agreement with the proposed value. The evidence strongly supports the validity of the proposed concept, and it is strongly believed that a fully adequate nutrient formula can be designed thereupon.

Herbert (9) studied the steady state continuous culture of aerobic bacteria in a system similar to ours with the exception that his cultures were not illuminated. When the nutrients were maintained in such excess that nothing required for growth was limiting, the plot of bacteria concentration as a function of feed rate became asymptotic to a line parallel to the abscissa axis as the feed rates decreased. This relationship does not hold for the continuous photosynthetic culture of algae. As shown in Figure 6, the plot of algal concentrations as a function of feed rate increases as the feed rate decreases. One possible explanation for the algae curve differing from the bacteria curve is light limitation. As the culture becomes denser light becomes limiting. Another possible explanation is that the kinetics of the photosynthetic process may be different from those of the respiratory process. It is appreciated that the culture of algae in our system was both a respiratory and photosynthetic process. However, the photosynthetic process was primarily responsible for algae growth as the only carbon sources in the medium other than carbon dioxide was urea and EDTA. Even if these carbon sources could support the heterotrophic culture of algae, their concentrations were too low for algal concentrations attained.

Summary and Conclusions

A completely mixed continuous steady system having a net volume culture of 2.7 liters has been developed using a high intensity light source (52,000 lumens) for the photosynthetic culture of algae. In the culture medium the concentrations of Chlorella 71105 were varied between 6.83 and 16.36 gm/l, dry weight basis, at respective feed rates of 201 and 67 ml/hr. A maximum daily yield of 36 gm/day was attained at an algal concentration of 8.98 gm/l with the feed rate maintained at 170 ml/hr.

A new concept for the design of the nutrient formula made the growth at high concentrations possible. This concept was based on supplying the minimum concentration of nutrients required for growth plus that required to produce algal cells.

Future Work

Five general concepts have been chosen for further study: more precise definition of the optimum illumination, mixing, and gas exchange characteristics of the system; study of the nutrient requirements of algae and their effect on algae as a food; a definitive study of human waste products as nutrients in the system; the advantages or disadvantages of symbiotic relationships between algal strains and between algae and bacteria, and the culture of other algal strains in the system. Work has been initiated on all five concepts. The four lamp system, which has been described, cannot maintain algal cultures free from bacteria contamination. Two new six lamp systems which are now being designed will allow sterilization of nutrient reservoir,

culture chamber, and harvest system as a single unit. These new units should maintain an algal culture of 4.2 liters under 198,000 lumen light intensity.

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Table 1. Mineral Composition of Chlorella 71105 Grown on T-4 Medium

Element	Percent Element of Dry Weight Algae
N	9.81
P	1.30
K	1.09
Na	Trace
Mg	0.524
Fe	0.0314
S	0.935
Cu	0.01078
Cl	0.146
Ca	0.0103

Table 2 T-4 Medium

Source of Nutrient Element	Conc. of Stock Sol. gm/L	ml of Stock/L of Medium	gm/L of Medium
$(NH_2)_2CO$			0.4
KH_2PO_4			2.5
$MgSO_4 \cdot 7H_2O$			5.0
$CaCl_2 \cdot 2H_2O$			0.0294
NaCl			2.0
$FeCl_3 \cdot 6H_2O$	2.416	1	
Trace #1 Elements			
H_3BO_3	2.858	1	
$CuSO_4 \cdot 5H_2O$	0.079	1	
$MnCl_2 \cdot 4H_2O$	1.801	1	
$ZnSO_4 \cdot 7H_2O$	0.2200	1	
MoO_3	0.0190	1	
Trace #2 Solution containing		10	
$MCr(SO_4)_2 \cdot 12H_2O$	0.0098		
NH_4VO_3	0.0023		
$NaWO_4 \cdot 2H_2O$	0.00179		
$KTi(C_2O_4) \cdot 2H_2O$	0.00740		
KOH	28.075		
EDTA	50		

Potassium hydroxide and acetic acid used for pH adjustment.

The main nutrient elements are in the same proportion as those recommended by Dean Burk to Electric Boat. However, a more complete trace nutrient formula than that recommended by Dean Burk has been used.

Table 3. T-6 Medium

Source of Nutrient Element	Conc. of Stock Sol. gm/L	ml of Stock/L of Medium	gm/L of Medium
$(NH_2)_2CO$			2.71
KH_2PO_4			3.055
$MgSO_4 \cdot 7H_2O$			5.6655
$CaCl_2 \cdot 2H_2O$			0.0335
K_2SO_4			0.1430
NaCl			2.0
$FeCl_3 \cdot 6H_2O$	3.416	7.875	
Trace #1 Elements			
H_3BO_3	2.858	2	
$CuSO_4 \cdot 5H_2O$	0.079	2	
$McCl_2 \cdot 4H_2O$	1.801	2	
$ZnSO_4 \cdot 7H_2O$	0.2200	2	
MoO_3	0.0190	2	
Trace #2 Solution containing		20	
$KCr(SO_4)_2 \cdot 12H_2O$	0.0098		
NH_4VO_3	0.0023		
$NaWO_4 \cdot 2H_2O$	0.00179		
$NiSO_4 \cdot 6H_2O$	0.00448		
$KTi(C_2O_4)_2 \cdot 2H_2O$	0.00740		
$Co(NO_3)_2 \cdot 6H_2O$	0.00495		
KOH	20.075		
EDTA	50		

Potassium hydroxide and acetic acid used for pH adjustment.

Table 4. Summary of Data

Run	Dates From	Dates To	Period hr	Feed Rate ml/hr	Dry Wt. gm/L	Daily Yield gm/day	PCV ml/10 ml	Range in pH
1	11-8-61	11-12-61	101	170	8.98	36.5	.33	6.4 - 6.5
2	11-18-61	11-24-61	133.25	82	13.60	26.5	.49	6.3 - 6.6
3	11-27-61	11-29-61	48	67	16.36	26.3	.56	6.3 - 6.8
4	12-2-61	12-3-61	--	56	21.08	29.4	.67	6.5 - 6.6
5	12-5-61	12-7-61	48	53	16.22	20.8	.55	6.5
6	12-9-6	12-11-61	48	53	14.87	18.9	.52	7.0 - 7.2
7	12-31-61	1-2-62	47	112	9.87	26.5	.37	6.6
8	1-15-62	1-18-62	71.75	111	11.33	30.1	.40	6.4
9	2-5-62	2-8-62	72.75	78	14.06	26.5	.50	6.4 - 6.5
*10	2-9-62	2-12-62	--	26.6	23.78	15.2	.79	6.3 - 6.4
11	2-27-62	3-1-62	47	120	10.77	31.0	.36	6.9 - 7.0
12	3-15-62	3-18-62	75.75	201	6.83	33.2	.26	6.5 - 6.7

*Dry weight based on single measurement when equilibrium was not established.

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