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HILIS - A HIGH INTENSITY LIGHT SYSTEM FOR ALGAE FOOD PRODUCTION

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In Japan today algae, grown in coastal waters much as grass is grown on land, sells as garnish for eight dollars a pound (dry weight) (1). The dense civilization of the Aztec Indians at the time of the Spanish conquest could probably not have existed except for the utilization of a bluegreen algae as food (2). From these two instances alone, it can be seen that the vanguard of food research lies not in the use of algae as a modern food but in the application of modern technology to finding practical economical methods for producing it. The algae which have been used as food are either filamentous or leaf-like in form. Such forms are easily harvested by manual methods but are not well suited to the unit operations of modern mass fermentation. As a consequence, the engineer and biologist have turned to the production of unicellular strains which are small rugged individuals (3 to 20 microns in diameter or maximum dimension) and have high growth rates. The Chlorella and Scenedesmus strains have been the most widely studied.

The selection of these strains required extensive study for wholesomeness. Results of these studies indicate that these strains with their natural symbiotic array of microflora could safely furnish 25% of a total caloric intake for man (3). As progress was made in strain selection, mass culture, nutritional evaluation, processing and food preparation, four problems became apparent: acceptability, nutritional quality, large culture volume requirements, and poor energy conversion efficiencies. With hundreds of thousands of strains to select from, with application of genetic technology and with processing methods already developed for flavor improvement (4), acceptability and nutritional quality offer no real obstacles. The problems of large culture volume requirements and poor energy conversion efficiencies are much more complex and formidable..

Mass culture under natural sunlight rarely yields concentrations as high as 1.5 g/L dry weight under purely photosynthetic

processes (5). The reasons for this are that sunlight is not continuous for 24 hours, intensity does not exceed 10,000 lumen and only a small portion of the radiant energy can be efficiently utilized by the photosynthetic processes. In our laboratory, batch systems utilizing fluorescent illumination at 2000 lumen produced 0.5 g/L/day. Early attempts to utilize high intensity illumination in excess of 10,000 lumen failed due to lack of understanding of the importance of light-dark phasing, nutrient requirements and heat transfer. Unfortunately, many erroneously concluded that the problem was one of a maximum permissible light intensity between 1000 and 2000 lumen. The successful operation of a four lamp (Quartzline) 2.7-liter continuous culture system (6, 7) demonstrated that intensities up to 52,000 lumen were feasible and that the dense culture of 9 g/L under continuous culture reduced the culture volume to 37.3 L/man as compared to 1000 L/man for the fluorescent batch system (based on a total food requirement of 500 g/day/man). Unfortunately, the geometric configuration of this early high intensity light system, though a breakthrough, would permit neither variation of growth parameters nor further increase in light intensity due to a problem of heat transfer. The same limitations apply to the system developed by P. J. Hannon and C. Patouillet (8) which is essentially a scale up of the four lamp system to six lamps. Comparisons of other systems are discussed by R. L. Miller and C. H. Ward (9).

Approach: A definitive study of the effects of variations in each of the environmental growth parameters was considered as serving several useful purposes: (1) reduce the culture volume, (2) improve the energy conversion efficiencies, (3) determine what, if any, effect a variation in the growth parameters might have upon quality and flavor of the food produced, and (4) describe the environmental growth parameters and their control thereby affording rational design criteria for scale up. The growth parameters include temperature, light intensity, light quality, light-dark phasing, gas exchange, heat transfer, availability of nutrients, pH, and maximum and minimum concentrations of specific nutrients. If these growth parameters could be controlled in one system then their interrelationships could also be determined. The high intensity light system (HILIS) was designed to do this. A description of the design, operation and function of the components to meet the study requirements will follow and the results of studies on the first three parameters presented. Results demonstrate a reduction in culture volume to 21.6 L/man and improved efficiency of 267% over the 4 lamp system.

DESCRIPTION OF HILIS

The HILIS will be described following the classical unit operations, processes and flow model concepts of chemical engineering indicating how these concepts permit a definition of the environmental parameters. Where applicable pertinent design considerations will also be discussed under each of these headings.

Figure 1 shows a schematic diagram of the principal functional components of the HILIS. Figure 2 is a photograph of the system less the heat exchangers and powerstat control to the lamps.

Illumination: Illumination is provided by six 1500 watt Quartzline lamps. Each lamp is jacketed with both a 22 mm and a 38 mm pyrex tube. Between these tubes chilled distilled water is circulated to absorb heat and to filter out ultraviolet and infrared light. Of the total energy input, 12% is emitted as visible light and 88% as energy at the ultraviolet and infrared wavelengths. The visible light is low in the blue and rich in the red wavelengths. For these lamps the ratio of blue at 440 nm to red at 680 nm wavelengths (wavelengths at which chlorophyll exhibits maximimum absorption) is 1 to 4.5. The use of filters was considered as a means of preventing the infrared light from reaching the culture and of striking a better balance between the blue and red spectrum, but was not found necessary. Operational procedures for maintaining temperature control and heat transfer were developed which eliminated the need for filtration of the infrared. Recent developments in the plasma generation of light with metal halide arc lamps make it possible to design a light source with a specific desired spectrum eliminating requirements for filters yet fitting configuration of lamp jackets.

The total illumination emitted from the Quartzline lamps is controlled through the regulation of the applied voltage using a powerstat. The total illumination may be computed by the following equation furnished by the lamp manufacturer where V is voltage and I is total illumination in lumens per lamp,

$$I = 33{,}000 \left(\frac{V}{277}\right)^{3.54}.$$

Heat transfer and temperature control: The design of the water jackets around the Quartzline lamps was determined experimentally based on heat transfer limitations. In the operation of an earlier high intensity system it was found that when the culture temperature rose at a rate of 1°C in 20 sec., the culture was adversely affected (6, 7). This rise in temperature was calculated to be an increase of 2000 calories per min. per lamp. Using this value as a limiting factor, several sizes of lamp jackets were tested, and a 38 mm diameter selected. It appears that a major portion of the infrared and visible spectrum pass successively through the inner 20 mm diameter pyrex tube surrounding the lamp, the distilled water layer, the outer 38 mm pyrex tube, and the medium where it is absorbed by the algal cells and converted into heat. This heat is then transferred by conduction through the surrounding medium, through the outer pyrex tube and back to the distilled water. Chilled distilled water is pumped to the lamp jackets and the heated water is returned to a heat exchanger for cooling. The use of copper sulfate for

absorbance of the infrared was tried but found to materially reduce the energy in the visible spectrum at concentrations effective for filtration of the infrared. The cooling water circulated around the lamps serves as the primary cooling system.

A 3/8" 304 series stainless steel tubing coil through which cold water is circulated on demand serves as the secondary cooling system. A solenoid value actuated through a temperature sensing device regulates the flow of cold water through the cooling coil.

The design approach was to circulate cooling water from a single heat exchanger through both the primary and secondary cooling systems. This was found to give too large a variation in temperature ($\pm$ 2.5°C). A procedure of using dual heat exchangers was worked out which permits a maximum temperature variation of $\pm$ 0.2°C from the mean. Water entering the lamp jackets is maintained at a maximum of 27°C while the cooling water delivered to the stainless steel coil is maintained at 4°C. The function of mixing in heat transfer is discussed below under the respective operation.

Safety temperature controls are an essential part of the equipment. An exterior surface mounted thermistor attached to the bottom flange sensesthe liquid temperature while a second surface mounted thermistor attached to the top flange senses overheating of the top portions due to an accidental drop in liquid levels below the lamps. These two sensing devices are wired in series to turn off the power to the lamps at preset maximum temperatures. Autoclavable thermistor probes are immersed in the liquid culture suspension to measure the culture temperature and to sense temperature for control of the secondary cooling system.

Gas transfer: Inlet gas flows from an air and carbon dioxide mixing facility through a metering valve, rotameter and Millipore filter to the culture vessel where it is injected through a 1/16" orifice directly below the center of the turbine impeller. A 1/16" stainless steel plunger is incorporated in the gas injection unit to facilitate cleaning the orifice without shutting down the system. Handling of outlet gases is described under flow model section.

Gas transfer, primarily from the standpoint of maintaining a supply of carbon dioxide to the organisms may be controlled by several methods: concentration of carbon dioxide in influent gases (air), flow rate of gas, and mixing.

Mixing: Mixing also serves as the means of controlling light-dark phasing, heat transfer, and availability of nutrients. While it is possible to determine relative importance to mixing upon each parameter, what is truly required is a definition of the effects of mixing variables upon the combination of the four

parameters in terms of net growth response. Light-dark phasing and heat transfer are flow dependent operations which give optimum results at low mixing speeds and large ratios of impeller to tank diameters (10). Gas exchange is a turbulence dependent operation which gives optimum results for high mixing speeds and small ratios of impeller to tank diameters. Nutrient availability is more than likely a function of both flow and turbulence. In the HILIS the rate of mixing may be varied from 0 to 1200 rpm and the impeller diameters from 1-1/2" to 6". The culture vessel has an equivalent diameter of 9.64 in. The culture vessel has an 8-3/4" square section with the corners beveled to improve the mixing configuration. The coils, piping, probes, and lamp jackets appear to serve as adequate baffles.

Flow model: Operation of the HILIS as a continuous steady state system will permit an accurate determination of the maximum and minimum concentration of nutrients and pH control. In a batch system the nutrient concentrations are continually changing from the time of seeding to the time of harvesting. In a continuous system, operating under steady state conditions, the nutrient feed rate is equal to the rate of harvest resulting in a constant concentration and composition of both nutrients and organisms. A change in concentration of a specific nutrient in the nutrient feed results in a change in concentration of that nutrient in the culture vessel. Any effect of a change in nutrient concentration is reflected directly by a change in the concentration of organisms in the culture and harvest.

Nutrient is fed to the culture chamber using a peristaltic pump, the rate being controlled by a timer. The harvest can be handled using an overflow weir tube or a pump controlled by an electronic level control. The overflow weir tube has been found to be the more dependable method of harvest. The harvest overflow tube also serves as the gas outlet. The liquid harvest suspension is trapped in a 20 liter carboy and the gas passes through a second trap followed by a 20% acid wash to reduce cross contamination in the laboratory. When the liquid harvest is electronically controlled, a separate gas outlet is provided from the top of the gas head space.

Sterilization: The HILIS was designed and built to permit in-place steam sterilization of all metal and glass components for pure culture work. Stainless steel of 304, 308 and 316 series has been used where contact with liquid and moist gases occur. Viton gaskets are used throughout for seals between metal parts and between glass and metal parts. Valving arrangements were devised to permit steam sterilization of pipe junctions between culture chamber and complementary vessels and equipment. For work with a single algal strain and its symbiotic microorganisms, primarily bacteria, a simpler valving arrangement has been used.

Foam control: An electronic system senses the presence of foam through a probe and actuates a peristaltic pump to deliver a limited quantity of antifoam. The period between additions may also

be controlled as desired by a timer.

ENVIRONMENTAL PARAMETER STUDIES

The basis for selecting the order of environmental parameters to be studied were: (1) independence from influence of other parameters, (2) influence upon the other parameters, (3) operational characteristics of HILIS, and (4) importance with respect to research aims. The planned sequence for the study of the parameters is as follows: (1) temperature, (2) light intensity, (3) mixing as an independent variable of gas exchange, light-dark phasing, heat transfer and nutrient availability, (4) light quality, (5) gas exchange, (6) determination of maximum and minimum concentration of nutrients, and (7) pH.

Work on temperature and light intensity have been completed. These completed studies and their significance are presented to demonstrate that the rationale of the approach is correct and that the HILIS can perform as designed. Chlorella 71105 was chosen for these studies as more is known about its physiology than any other alga and has served as the model organism in our previous studies. As the Chlorella 71105 with the natural occurring symbiotic microorganisms were used for these studies, aseptic techniques were not followed. A selection of operating conditions within the limits of the environmental conditions for algal growth was made based on previous experience in operation of earlier illuminated fermentors and actual experimentation during the long period required for the adjustment of mechanical and electrical functioning units. The conditions set for these studies have been summarized in Table 1.

Temperature parameter: Under low intensity light (less than 5000 lumen) the optimum temperature for growth for Chlorella 71105 has been determined to be 39°C. As explained earlier, the problem of heat transfer under very intense illumination is a complicated one. The algal cells as they pass the light source are undoubtedly hotter than the surrounding medium and are nearly as cool as the medium as the cells pass by the secondary cooling coil. To place the temperature sensing probe at the correct point to measure the precise average temperature of the cells or the medium would be a major undertaking of little value with relation to the immediate aims of the project. The same end was more easily attained by placing the sensing probe in a position least affected by either the light source or the cooling systems and then measuring growth response at specific measured temperature to obtain the range for optimum growth. The values for dry weight, packed cell volume and cell count gave curves similar to that shown in Figure 3 for the dry weight versus temperature. A study of the curves indicate that good growth occurs within a range of 35°C and 39°C with a maximum growth occurring at 35°C. A T-6 medium was used for this study (6, 7). As cell concentrations at 200,000 lumen and 35°C were high (18.2 g/l), there was concern that nutrients might be

limiting growth. A new T-30 medium was designed to support 30 g/l following a procedure described in a previous publication (6, 7), and was tested under the same conditions as the T-6 medium. As no increase in dry weight, packed cell volume, or cell count was encountered using the T-30 medium, the nutrients were not considered to be limiting growth.

Light Intensity: Operating at 35°C, the effect of light intensity upon growth was studied. Results are given in Table 2. A plot of this data (Figure 4) indicates that total yield in g/day dry weight may be expressed as a linear function of the log of total illumination in lumens when feed rate is constant.

It appears that the maximum values for cell concentration and total illumination have not been reached at the largest measured value of 25.5 g/l dry weight under 300,000 lumen (this illumination is equivalent to 30 times the intensity of sunlight as measured over a square foot of the earth's surface at noon). At a total illumination of 300,000 lumen, the surface intensity at the outside of the 38 mm jacket is 189,000 lumen. This intensity is 90 times greater than the value of 2000 lumen used in the batch system in 1960. An arithmetic plot of the data shows that the optimum utilization of light energy occurs in a range between 100,000 and 200,000 lumen total illumination under the environmental conditions studied. This is not the ultimate; it is but the first approximation. Before any meaningful attempt at precise definition of total illumination and light intensity can be attempted, the other environmental conditions need be brought into the ranges for optimum growth. These results do offer a basis for predicting the effect of total illumination upon growth and demonstrate that neither maximum intensity nor maximum total illumination have been reached.

In a practical sense, the results are even more encouraging. The first aim of this research has been achieved with a major reduction in volume requirements. In 1961, 400 liters were considered a basis for the design of algal systems to meet the food and gas exchange requirements of one man. The present attainment demonstrates that only 21.6 liters are required (based on 500 g/man/day intake).

With respect to the second aim there has been a significant increase in energy conversion efficiency over our earlier work. A comparison of the results of the HILIS with the earlier 4 lamp high intensity system (6, 7) may be made on the basis of total energy input measured as lumens of visible light when the dilution rates are equal. On the basis of total energy at 52,000 lumen, total daily yields from the HILIS are 262% of those of the 4 lamp system.

FUTURE

When all nine environmental conditions can be brought into range for optimum growth the volume requirements are expected to approach 5 liters per man. At this time overall energy conversion efficiencies in converting electrical energy into a high energy biomass will probably approach 40%. High energy conversions will be attained through matching light sources with light requirements. Initial studies on light quality offer exciting prospects of a major breakthrough in energy conversion when coupled with present-day attainments by lamp manufacturers in plasma generation of light. The metal halide arc lamps are 5 times as efficient as incandescent lamps and, in addition, permit selection of desired wavelengths.

Light quality: Although much work has been reported as to the effect of certain narrow band spectra upon the photosynthetic process, none of it could be correlated to permit even a reasonable selection of specific monochromatic lights, or their relative energies for the design of light quality experiments in the HILIS. Work was conducted, under contract, by Dr. Thomas E. Brown of The Charles F. Kettering Institute to make a definitive study of the monochromatic light requirements of 17 strains of algae representing ten taxonomic divisions and known differences in photoactive pigmentation (11). Each strain was individually grown at equal energy levels in white light and light of nine separated 10 nm bandwidths corresponding to the major absorption peaks of known pigments. Measurements were made of growth, pigmentation, photosynthesis, respiration, and where possible, morphology and structure. These studies indicate: (1) while light of one specific wavelength is required for the formation of a pigment, usually another wavelength is necessary for the efficient utilization of the pigment in oxygen production, (2) broad spectrum light is many times more important in pigment formation than in pigment utilization, (3) light specificity has a significant effect upon the respiration of organisms which are predominately heterotrophs (this effect is primarily a function of light adaptation), and (4) there is a synergistic effect resulting from compounded wavelengths. These results not only offer a firm basis for wavelength selection based on pigment action but suggest methods for controlled ripening of green fruit at time and place of use, control of growth of heterotrophic organisms, and control of mode of reproduction in algae.

SUMMARY

Under the mass culture phase two problems have presented major obstacles to the ultimate establishment of a practical culture system - large culture volume requirements and poor energy conversion efficiencies. An investigation of the parameters influencing growth has been the approach to eliminating these obstacles. The HILIS was designed as a functional experimental system to permit the definition of the environmental parameters for optimum production.

of an algal biomass offering rational engineering design criteria basic to scale up. These parameters include temperature, light intensity, light quality, maximum and minimum concentration of nutrients, light-dark phasing, gas exchange, heat transfer, availability of nutrients, and pH. Results of light intensity studies demonstrate that the first goal - high yield in a small space - is attainable. The HILIS has been operated as a continuous system with a dilution rate of 0.91 per day (7L/day) under 300,000 lumen of incandescent illumination (30 times intensity of sunlight) yielding 25.5 gm/L dry weight of Chlorella 71105. The increased culture density attained through light reduces the culture volume required for the support of one man from the often stipulated 400 liters to 21.6 liters.

Initial results in light quality parameter studies make it possible to select the spectrum for maximum utilization of light by an algal strain. This, coupled with plasma generation of light offer prospects of a major breakthrough for greatly increasing the energy conversion efficiencies.

These initial attainments not only confirm the rationale of the approach to define the environmental parameters in one system but also demonstrate the means for doing so. It would appear that the HILIS is to become a prototype of systems for the mass culture of algae, a food of the future.

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Table 1 - Conditions for Operation of HILIS for temperature and light intensity parameter studies.

Condition	Units	Setting for Study of Temperature	Setting for Study of Light Intensity
Total Illumination	Lumen	200,000	25,000 to 300,000
Gas Flow Rate	l/hr	60	60
Conc. of CO_2 in Air	% of total	10	10
Impellar Dia.	inches	4	4
Impellar/tank	diameter ratio	.43	.43
Impellar Speed	rpm	450	450
Nutrient Feed Rate	ml/hr	284 ± 5	295 ± 10
Dilution Rate	per day	~0.9	0.91
Temperature	°C	31 to 40	35
Organism		Chlorella 71105	Chlorella 71105
Nutrient (6)		T-6 & T-30	T-6
Primary cooling inlet	°C	~ 21	21
Secondary cooling inlet	°C	4	4

Steady State Condition - Above conditions maintained with ± 0.2 gm/L dry weight for a minimum period of 48 hr.

Table 2 - Summary of data taken measuring growth as a function of light intensity at dilution rate of 0.91/day in HILIS.

Total Illumination	Run	Length Run	Dry wt.	PCV	Total Yield
Lumens		hours	g/l	ml/ml	g/day
25,000	1	72	5.20	.021	36.4
	2	48	5.25	.021	
	3	72	5.15	.021	
50,000	1	91	9.8	.039	68.6
	2	48	9.8	.038	
	3	192	9.7	.038	
100,000	1	96	15.6	.052	107.8
	2	72	15.4	.051	
	3	72	15.7	.051	
200,000	1	264	19.8	.092	138.6
	2	115	19.8	.093	
300,000	1	144	25.5	.132	178.5

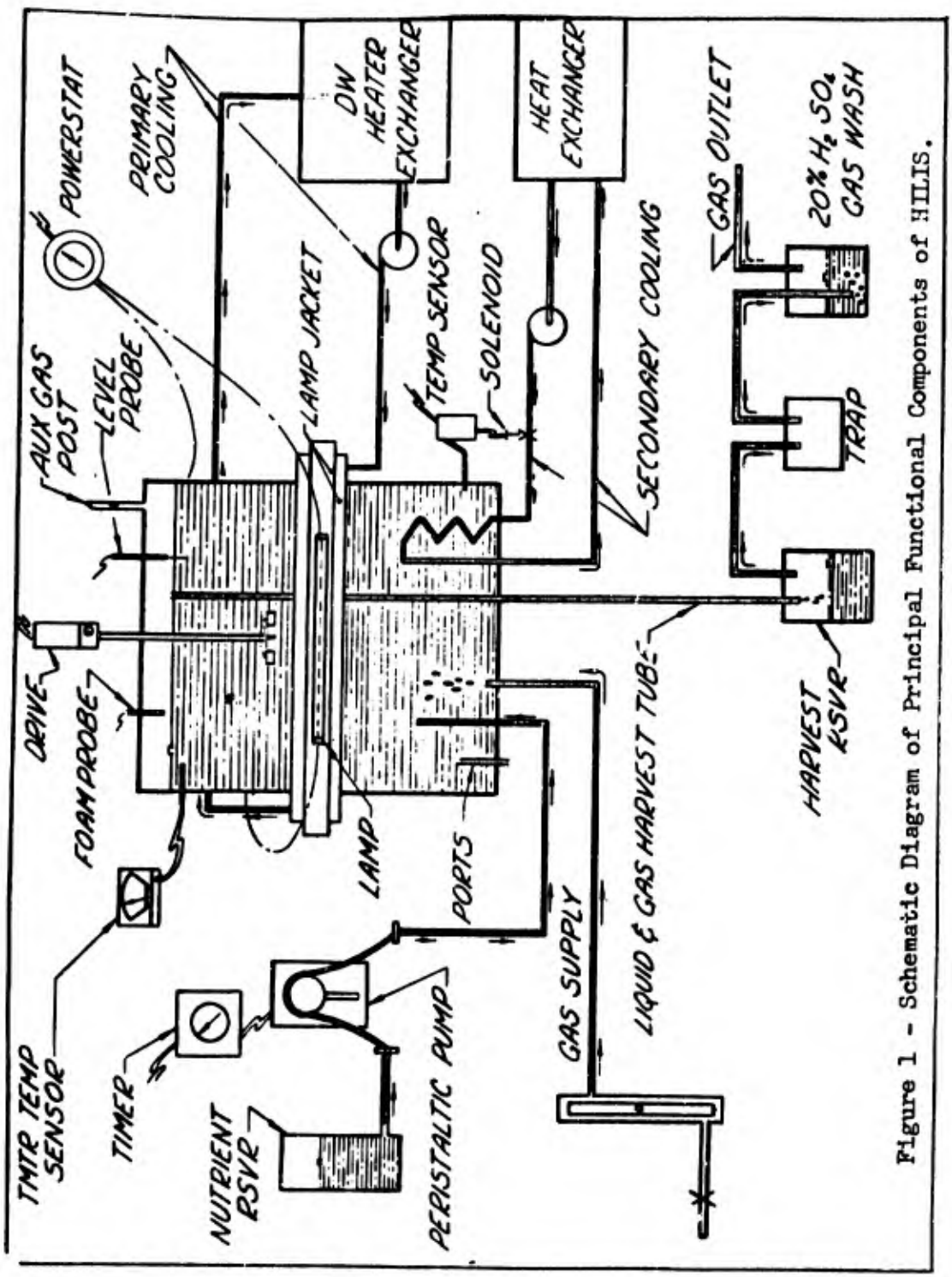

Figure 1 - Schematic Diagram of Principal Functional Components of HILIS.

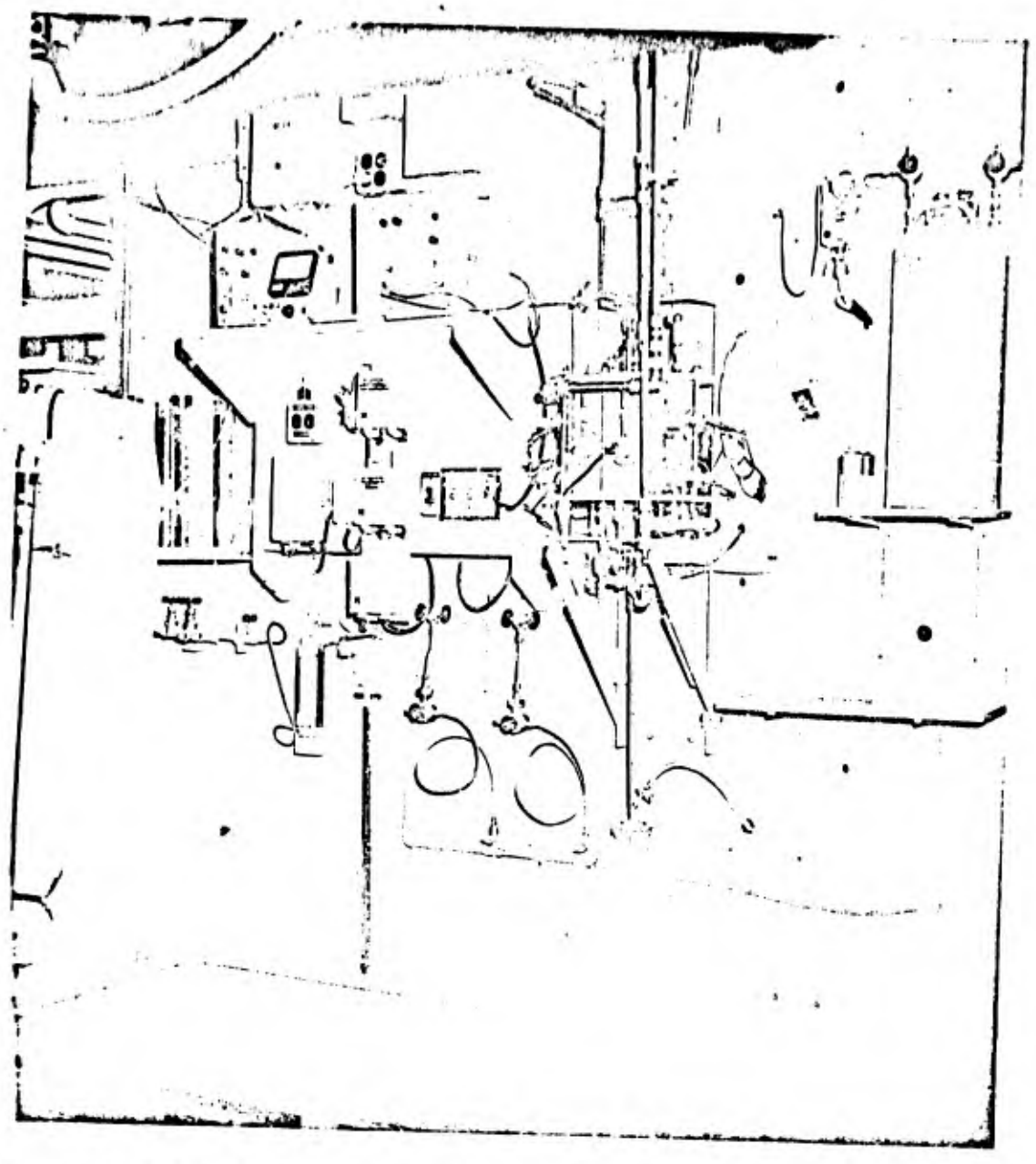

Figure 2 HILIS (High Intensity Light System) set up for continuous culture of Chlorella 71105. On left side are all electronic and electrical switching, antifoam, temperature indicator, level, timers, and temperature relay controls mounted on offset panel below which is gas metering and filtration line. In center, from top to bottom are variable drive to impeller, culture chamber and harvest reservoir. On right are nutrient reservoir and peristaltic pump. Heat exchangers, gas trap and wash, and powerstat to lamps are not shown in photograph.

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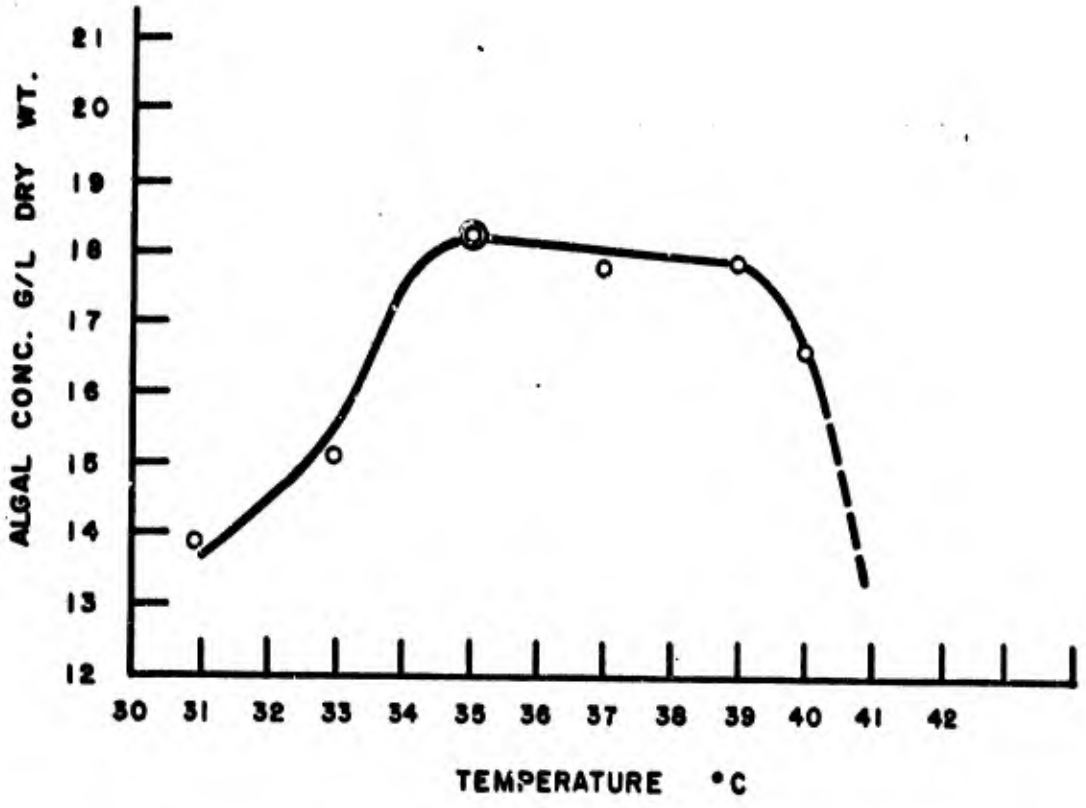

FIGURE 3 - Effect of Temperature on Growth of Chlorella 71105 in HILIS

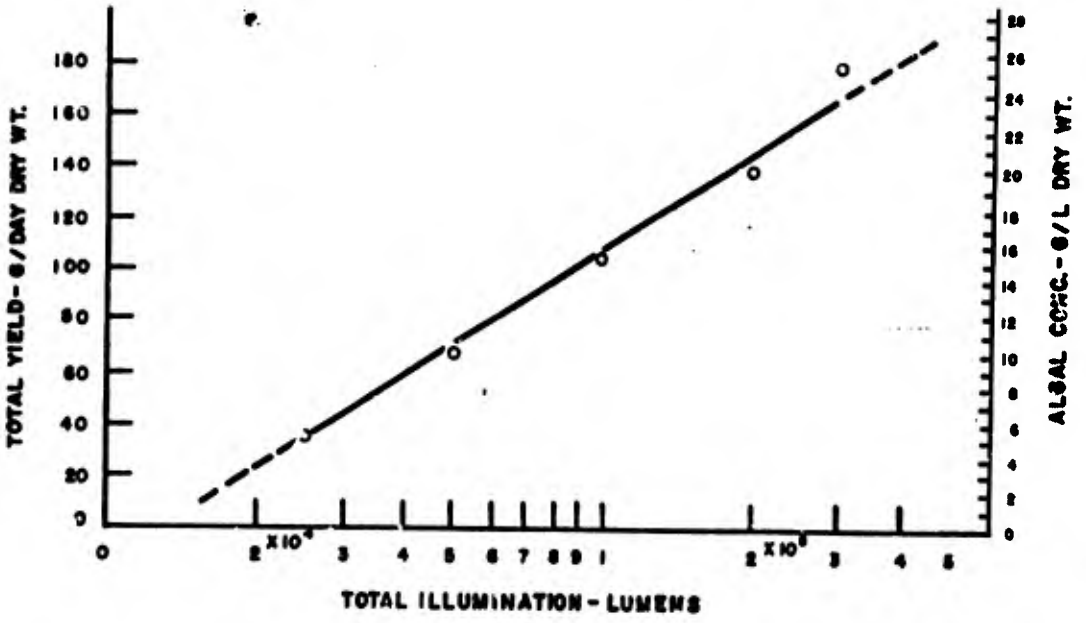

FIGURE 4 - Effect of Illumination upon Production of Chlorella 71105 During Continuous Operation of HILIS, Dilution Rate 0.91/Day