# A CHEMOSTATIC STUDY OF THE ASSIMILATION OF NUTRIENTS BY *CHLAMYDOMONUS RUGOSA* AT VARIOUS NITROGEN TO PHOSPHORUS CONCENTRATION RATIOS

by

PHILIP DAVID RICE

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## ABSTRACT

*Chlamydomonus rugosa* does not readily grow when transferred to a medium of different concentrations of elements unless N and P are in high concentrations. Chemostat reactors should be accessible from the top to facilitate the experimental procedure. A G. E. exposure meter (model 8DW58Y4) can be used to estimate light intensity. One foot candle registered on the meter, when the lid is closed, is equivalent to $1.13 \times 10^{10}$ ergs/$m^2$/hr.

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## Introduction

The use of continuous flow (chemostat) systems when culturing organisms has become increasingly popular over the last 15 years (Provisional Algal Assay Procedures, 1969). Continuous flow studies allow steady state growth rates. Under laboratory conditions, one can measure the steady state standing crop of a biological population that is sustained at a particular concentration of a rate limiting nutrient. As with batch culturing techniques, light intensities and spectral quality, temperature, and other pertinent variables, can also be controlled.

This study defines the rate of growth of algae under varying N and P concentrations that are found in fresh water lakes. By studying the uptake of these two nutrients in a chemostat for waters of different N to P ratios, the influence of one nutrient on the uptake of the other can be ascertained.

A continuous flow culture system was set up to simulate the environment found in natural fresh water (ie. Lake Ontario) at a depth of 5 meters. The temperature, light intensity and spectral quality, photosynthetic period, and the bulk composition of the culture media were all regulated within limits that are readily encountered in fresh water lakes at this depth. The uptake relationships incurred at low nutrient concentrations were of particular interest.

## Previous Work

Ketchum (1939), using batch cultures, found that the phosphate uptake by Nitzchia closterium (a salt water diatom) was dependant on the phosphate and nitrate concentration but the nitrate uptake depended on the nitrate concentration alone, when all other conditions were held constant.

Since Ketchum's work many investigators have examined algal physiology in chemostat studies but no one has redone the work of Ketchum in a steady state environment. Previous culturing techniques and results are discussed as follows.

### Algae

The use of a single species culture reduces the variables such as competition, predation, and differential nutrient uptake that would be incurred if natural diversity was allowed. Rarely are natural waters inhabited by one species. In order to quantify the differential uptake of N and P at various N to P ratios, one species must be used to eliminate preferential uptake of N or P by each algal species which which could mask the influence of other nutrients on the assimilation of one nutrient. The use of homogeneous algal cultures will remove the natural interaction of heterogeneous systems but interpretation of the uptake will be simplified.

The presence of bacteria in the medium can deplete substrates such as glucose and acetate (Hobbie and Write, 1966) resulting in the retardation of growth of heterotrophic algae. On the other hand, bacteria will decompose organic material thereby releasing $CO_2$.

(Kuentzel, 1969) and organic molecules to the algae. In environments where algal growth is limited by low $CO_2$ concentrations the presence of bacteria may promote algal blooms.

The use on non-filamentous, round, single celled algae has been recommended by Stadelmann (personal communication). Planktonic green algae will not grow on the inside walls of the culture vessels and hence more incident light will be able to reach the interior of the reactors. If the algae studied constitute an important fraction of the total biomass in fresh water lakes, then experimental results will have more significance when applied to behavior in natural waters.

When selecting an algal species for study its nutritional requirements must be carefully considered. Hopkins and Wann (1926) have found that Ca is not essential for growth of Chlorella. Greenfield (1942) notes that Cu concentrations of $10^{-7}$ molar may inhibit photosynthetic processes in some species. Many algal strains require vitamin $B_1$ (thiamine), vitamin $B_{12}$ (cobalmine) and biotin (Holm-Hausen, 1969) before satisfactory growth can occur. Chlamydomonus may not form gametes (Robbins et al, 1957) if the right combination of light coupled with a nitrogen-carbohydrate balance is not present. Reviews of nutritional requirements of algae are given by Ketchum (1954) and Lewin (1962). Prior knowledge of any idiosyncracies in the nutritional requirement of the algae under study will enable the proper culture media to be used so that no unknown substrate will be limiting.

Consideration must be given to the method in which algae assimilate nutrition. Some strains of Euglena and Chlamydomonus

(Schere, 1969) grow equally well autotrophically in light as they do heterotrophically in the dark. Algae with physiologies such as these can invalidate certain assimilation studies because of their dual uptake capabilities.

If the study is not designed to examine the consequences of growth of a particular species, then it is best to choose an algal culture for which there is extensive reference material (eg. Chlorella) enabling background physiological information to be obtained. Chlorella, along with many other species of algae, may be obtained from the Curator of the Culture Collection, Indiana University, Bloomington Indiana.

Once the species of algae has been chosen, it can be stored at room temperature under approximately 65 foot candles of light (Glooshenko, personal communication). The room lights should be switched off for half of the day thereby simulating a 12 hour photosynthetic day.

Measured in situ rates of uptake, in fresh water lakes, by algal populations of 2000 cells/ml. for N, P, and C are: 50-100 ug $N/m^2/hr.$, 1-10 ug $P/m^2/hr.$, and 50-100 ug $C/m^2/hr.$ respectively (Harris, personal communication). In chemostat studies nutrient uptake should approach the above assimilation rates but deviations will occur depending on the species of algae used.

Culture Media

When selecting a culture medium, consideration must be given to 1) the major ions, 2) trace elements, 3) nutrient concentrations, 4) the pH, 5) organic material in solution, 6) toxic substances, and

7) the stability of the constituents. The major ions consist of K, Ca, Mg, Cl, Na, and $SO_4$. Diatoms require Si. Trace elements that are usually required include Fe, Zn, Mo, Mn, Cu, Co, and V. Carbon, nitrogen, and phosphorus are found in atomic ratios of approximately 100 : 20 : 1 in algal cells. These can be supplied to the organisms as $CO_2$, $HCO_3$, $CO_3$, glucose, ortho- or pyro-phosphate, nitrate, ammonia, nitrite, urea, etc. Variation in the pH will change the rate of assimilation of nutrients by the algae and the state in which the nutrients are present (ie. $CO_2$ concentrations vary with pH). Organic molecules found in natural water are essential before some strains of algae will grow. The addition of vitamin $B_{12}$ complexes or yeast extract will provide these ingredients. The sources of nutrients and their stability must be considered in light of the physical conditions that the system is going to be subjected to. Urea, for example, is partially hydrolysed if autoclaved (Myers, 1962).

If air is bubbled through the system high algal populations may reduce the C concentration to values lower than those of a natural environment, causing it to be limiting. Most workers aerate their culture media with a 5% $CO_2$-95% air mixture to prevent C depletion. Myers (1962) suggests that air mixtures enriched by 0.5% $CO_2$ is adequate. Brown and Arthur (1969) found no difference in the total productivity when air enriched with 4% $CO_2$ was bubbled through the culture vessels but the rate of growth was slightly faster for the the enriched air mixtures (when compared to air mixtures).

Hume (1967) has found that labroratory distilled water may contain concentrations of Pb, Cd, and Cu that are only an order of magnitude below levels that can inhibit photosynthesis

(Greenfield, 1942). Therefore any addition of these "toxics" from other sources (eg. contamination in the compounds used for some artificial media) may increase their concentrations to growth limiting magnitudes. The use of distilled water that is free of high trace metal concentrations will eliminate possible toxic levels of minor ions in the culture media.

In order to minimize changes of water chemistry (Algal Assay Procedure: Bottle Test, 1971) prepared samples should be kept cool ($4^{\circ}$ C) and dark, preferrably without an overlying air space in a stoppered container. The shorter the storage period the less the chemistry of the solution will alter.

The synthetic culture media used by the Canada Centre for Inland Waters (CCIW) is given in appendix 1. It promotes healthy algal blooms but its elemental concentrations are not comparable to natural fresh water lakes. Other successful culture media that have been used are referred to by Ketchum (1954).

Light Intensity and Spectral Quality

The intensity of sunlight at any one point varies from dawn to dusk. Figure 1 shows the empirical "sine" relationship existing between the intensity of light penetrating 5 meters of water and the time of day.

At 5 meters of depth in L. Ontario the intensity of light varies between 2 and 5 $x10^{11}$ ergs/$m^2$/hr. (Jerome and Stadelmann, personal communication). Fifty percent of the radiation reaching the surface of a natural water body is in the visible range (figure 2). As light penetrates through the water, selective absorption of

the blue and red ends of the spectrum leaves significant energy only in the blue-green region of the visible spectrum (figure 2).

Successful culture experiments have been preformed under 500-1000 foot candles (Bain, 1969), 860 foot candles (Porcella, 1969), 170 foot candles (Pearson et al. 1969), and 350-400 foot candles (Brown and Arthur, 1969).

Oswald and Gaonkar (1969) have found that 30 to 40 days of an alternating 12 hour light, 12 hour dark environment is equivalent to 10 days of continuous lighting. Simulation of natural lighting conditions might therefore extensively prolong the duration of the experiment.

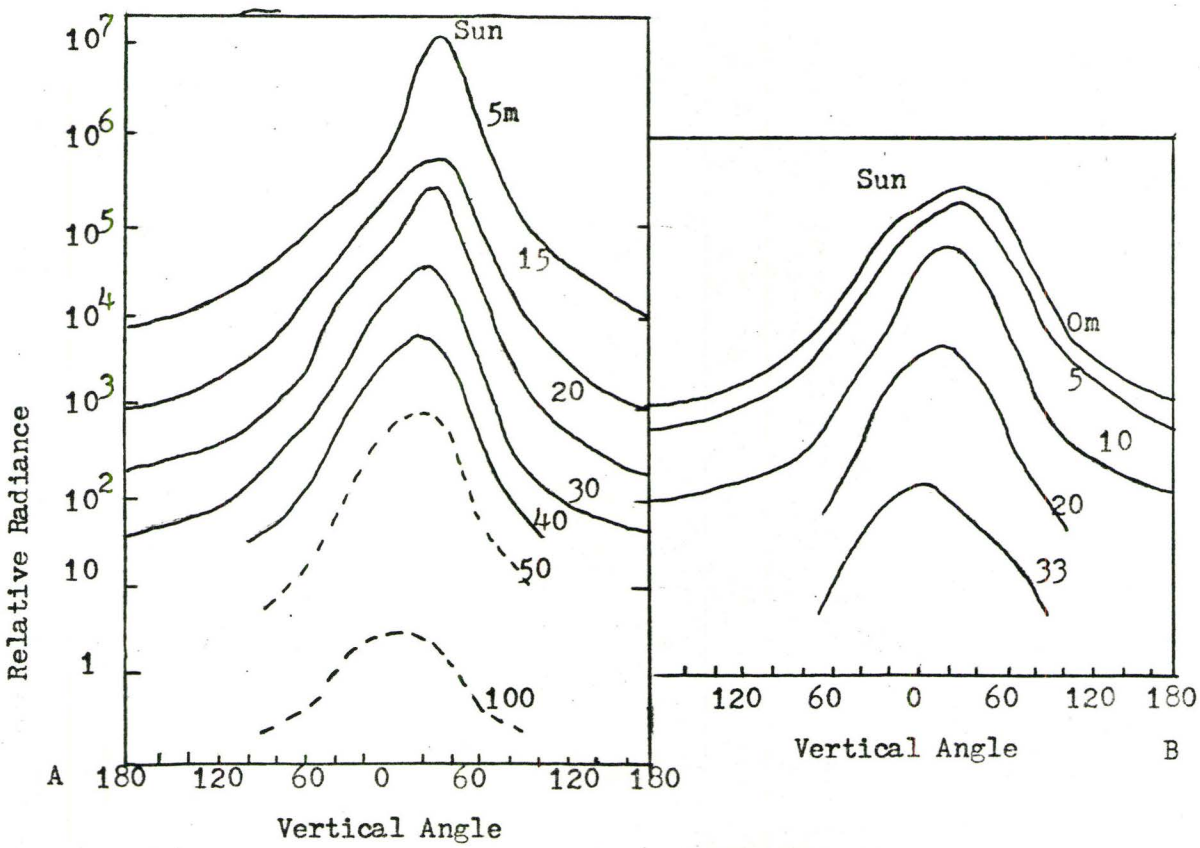
Harris, Glooshenko,(personal communications) and Maddux and Jones (1964) advocate the use of light sources containing the red wavelenghts of the visible spectrum. Light of this quality is not found at depths in natural water but it does appear to increase algal growth.

Temperature

The Provisional Algal Assay Procedure (1969) stipulates the rigid control of the culturing temperatures. Oswald and Gaonkar (1969), however, have found that this may not always be necessary. Their studies reveal that Chlorella grew equally well between the temperatures of 18° C and 25° C. Above and below these temperatures growth was less efficient. Work by Sorokin (1959) has revealled a maximum growth temperature of 42° C for one species of Chlorella. Most species will have a particular temperature range in which they will grow more rapidly. When modelling natural systems, however, the temp-

Figure 1

Change of the Suns Radiance at Different Vertical Angles (ie time of day)



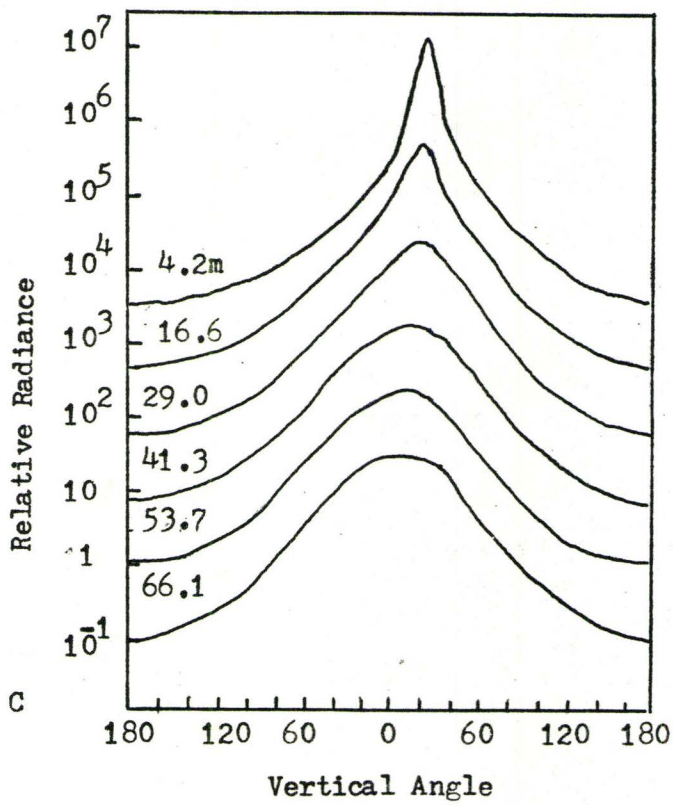



Figure 2

Schematic Representation of the Energy Reaching the Surface at Various Depths in Pure Water

(after Sverdrup et al., 1942)

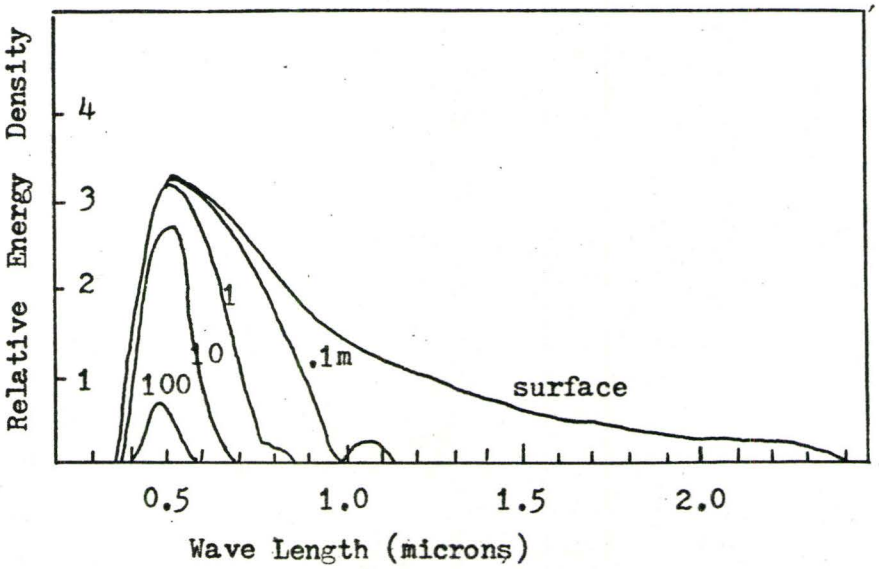



Figure 1: Change with increase of depth of radiance in the vertical plane of the sun towards the asymptotic state. A. Baltic Sea, green light (continuous line, after Jerlov and Liljequist, 1938) B. Gullmar Fjord, blue light and C. Lake Pend Oreille, green light (after Tyler, 1960).

erature should be restrained within the natural temperature ranges that are encountered in fresh water lakes.

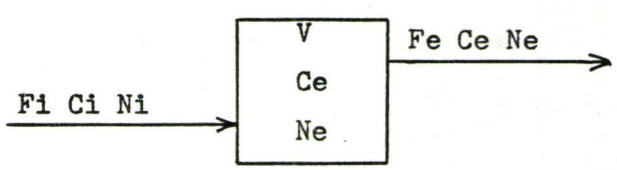
Chemostat Design

The chemostat should be built so that component parts can be disassembled and easily autoclaved where necessary. Various chemostat designs have been illustrated in the literature (Myers and Clark, 1944; Makel and Fencl, 1966; Maddux and Jones, 1964; Provisional Algal Assay Procedure, 1969; and Pearson et al, 1969). Design of the reactors can be variable but vessels incorporating inherent restrictions on light penetration, flow rates, media mixing and temperature control should not be used. Brown and Arthur (1969) found that partially filled 500 ml. flasks made the best reactors. Peristaltic pumps are usually used to regulate the flow of nutrient media through the chemostat. Carpenter (1968) has developed a simple inexpensive chemostat ($25 per reactor) with flow rates ranging from 6± 0.3 to 20± 0.4 ml/hr. by incorporating an electrolysis pump into his design. Where possible glass tubing should replace plastic or rubber tubing in order to reduce the amount of adsorption by the tube walls (Glooshenko, personal communication).

Chemostat Theory

Malek and Fencl (1966) and Fogg (1965) have reviewed the operation theory of continuous flow culturing devices such as the model shown schematically in figure 3.

Figure 3



where Fi is the influent flow

Fe is the effluent flow

Ci is the concentration of cells fed into the system

Ce is the concentration of cells in the effluent and reactor

Ni is the initial concentration of the limiting nutrient

Ne is the concentration of the limiting nutrient in the effluent

Since the volumn (V) of the reactor is constant, Fi=Fe=F

If no algae are added to the system in the influent then Ci=0. This will necessitate an initial seeding of the algae directly into the reactors at the start of the experiment.

The growth rate is a function of the concentration of the limiting growth rate and the biomass in the reactors which are related by the Michaelis-Menten equation as follows:

$$u = \frac{u_{max}\, Ne}{Kn + Ne} = \frac{dCe}{Cedt} \qquad 1$$

where u is the growth rate

$u_{max}$ is the maximum growth rate

Ne is the growth limiting nutrient concentration

Kn is the constant (determined in figure 4 or 5)

Ce is the concentration of algae in the reactor

t is the time

If the yield of algae and the ratio of live to dead organisms are constant then the change of algal concentration in the reactors is equal to their growth minus their decay and washout rates.

$$\text{ie } \frac{dCeV}{dt} = uCeV - KdCeV - FCe$$

at steady state $\frac{dCe}{dt} = 0$

and $$uCeV - KdCeV - FCe = 0$$

or $$(u - Kd)CeV = FCe$$

or $$u - Kd = F/V$$

where Kd is the decay constant

Since V/F is the hydraulic residence time, $\theta$, then

$$u - Kd = 1/\theta \qquad 2$$

When equation 2 is substituted into equation 1, then

$$1/\theta + Kd = \frac{u_{max}\, Ne}{Kn + Ne}$$

which simplifies to

$$Ne = \frac{Kn(1/\theta + Kd)}{(u_{max} - 1/\theta - Kd)} \qquad 3$$

Since $u_{max}$, Kn, and Kd are characteristic of an algal species, the limiting nutrient concentration (Ne) is only a function of the hydraulic residence time ($\theta$). As $\theta$ is increased Ne is reduced. The data for a chemostat experiment can be obtained by varying the volumes of the reactors or varying the flow rates.

If the input of nutrients to the system by decaying organisms is neglected, then the change of growth limiting nutrient in the reactors is equal to the input less the output and uptake, ie

$$\frac{dNeV}{dt} = FNi - FNe - \frac{dN}{dt} \qquad 4$$

Y is the yield of cells produced per amount of limiting nutrient available, ie

$$Y = \frac{Ce - Ci}{Ne - Ni} = \frac{dCe}{dN} \quad , \quad \text{since } Ci = 0$$

Recall that $\frac{dCe}{dt} = uCe$, therefore

$$\frac{dN}{dCe} \frac{dCe}{dt} = 1/Y \times uCe$$

so that equation 4 becomes

$$\frac{dNeV}{dt} = FNi - FNe - \frac{uCeV}{Y}$$

At steady state $\frac{dNe}{dt} = 0$, and $F(Ni - Ne) = \frac{uCeV}{Y}$

or $Ce = \frac{F}{V}(Ni - Ne)\frac{Y}{u} = \frac{(Ni - Ne)Y}{\theta u}$ 5

If growth rate is a function of the limiting nutrient and it is assumed that the growth rate is regulated by the rate at which the limiting nutrient is assimilated (q), then

$$u = Yq \qquad 6$$

Substituting equation 6 into equation 2

$$1/\theta = Yq - Kd \qquad 7$$

To solve for equation 1 figures 4 or 5 may be used. Equation 7 can be solved using figure 6

By conducting a series of three or more steady state experiments at different flow rates the system constants $u_{max}$, Kn, Y, and Kd can be calculated from the measured parameters F, Ni, Ne, Ce, and $\theta$. Kd and Y can be calculated from figure 6 where one steady state experiment will give one data point. Equation 2 or equation 6 (ie $u = Kd + 1/\theta$ and $u = Yq$ respectively) can be used to calculate u from the graphical results obtained from figure 6. Each steady state experiment will yield one u and Ne value which can now be plotted on either figure 4 or 5 to obtain values for the constants $u_{max}$ and Kn.

Figure 4

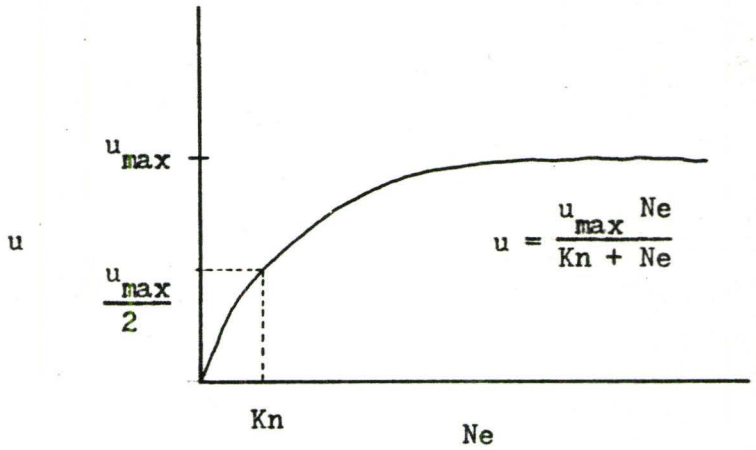



Figure 5

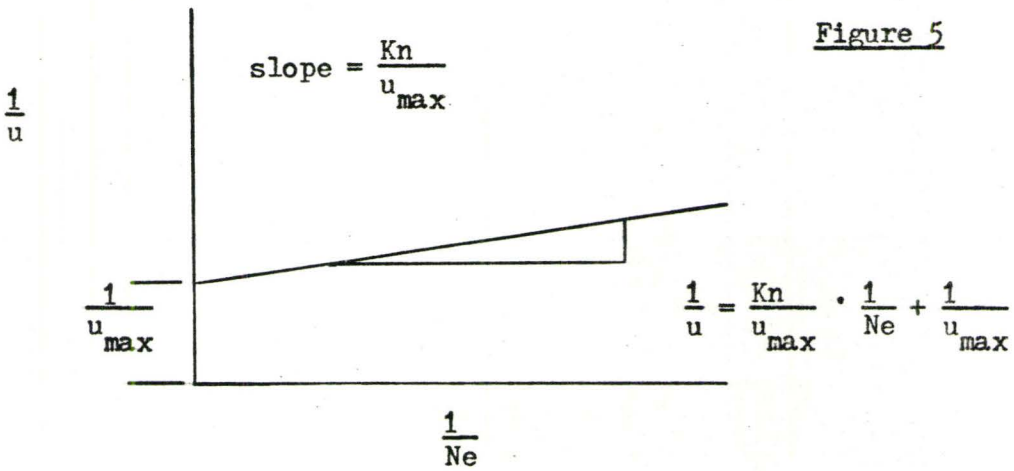
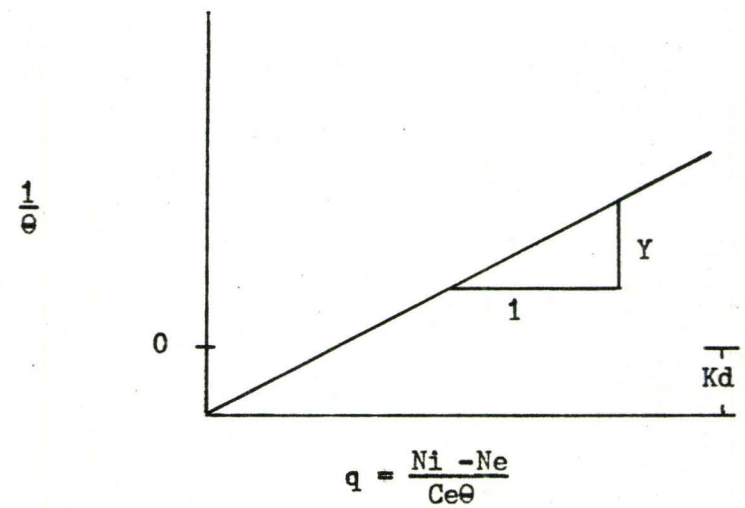



Figure 6



## Experimental Procedure

### Apparatus

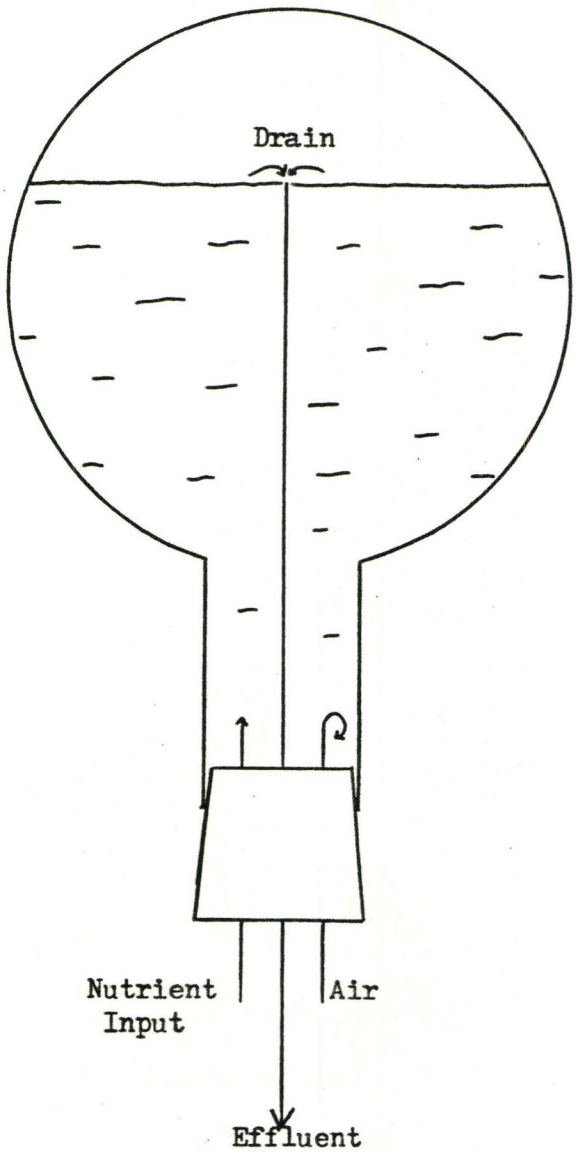
#### Chemostat Reactors:

The four chemostat reactors ( figure 7) were inverted 500 ml. round bottomed flasks, chosen in order to minimize internal reflections that might arise in irregularly shaped vessels. The nutrient and air mixtures were introduced through separate channels to the base of the culture media so that their circulation would resuspend any plankters that settled out. The effluent was drained from the top of the nutrient solution to maximize the mixing of the influent. The drain height allowed each reactor to hold 410 $\pm$ 10 ml. of culture media. The overflow was collected in 1 litre light excluding containers. The different nutrient solutions were pumped in at a constant rate of .02 $\pm$ .01 cu. ft./hr., setting up a slow circulation cell within the culture media. One in line glass wool filter and two in line water baths removed any impurities (oil etc.) from the compressed air and increased its vapour pressure of water (figure 8).

#### Light Intensity and Spectral Quality:

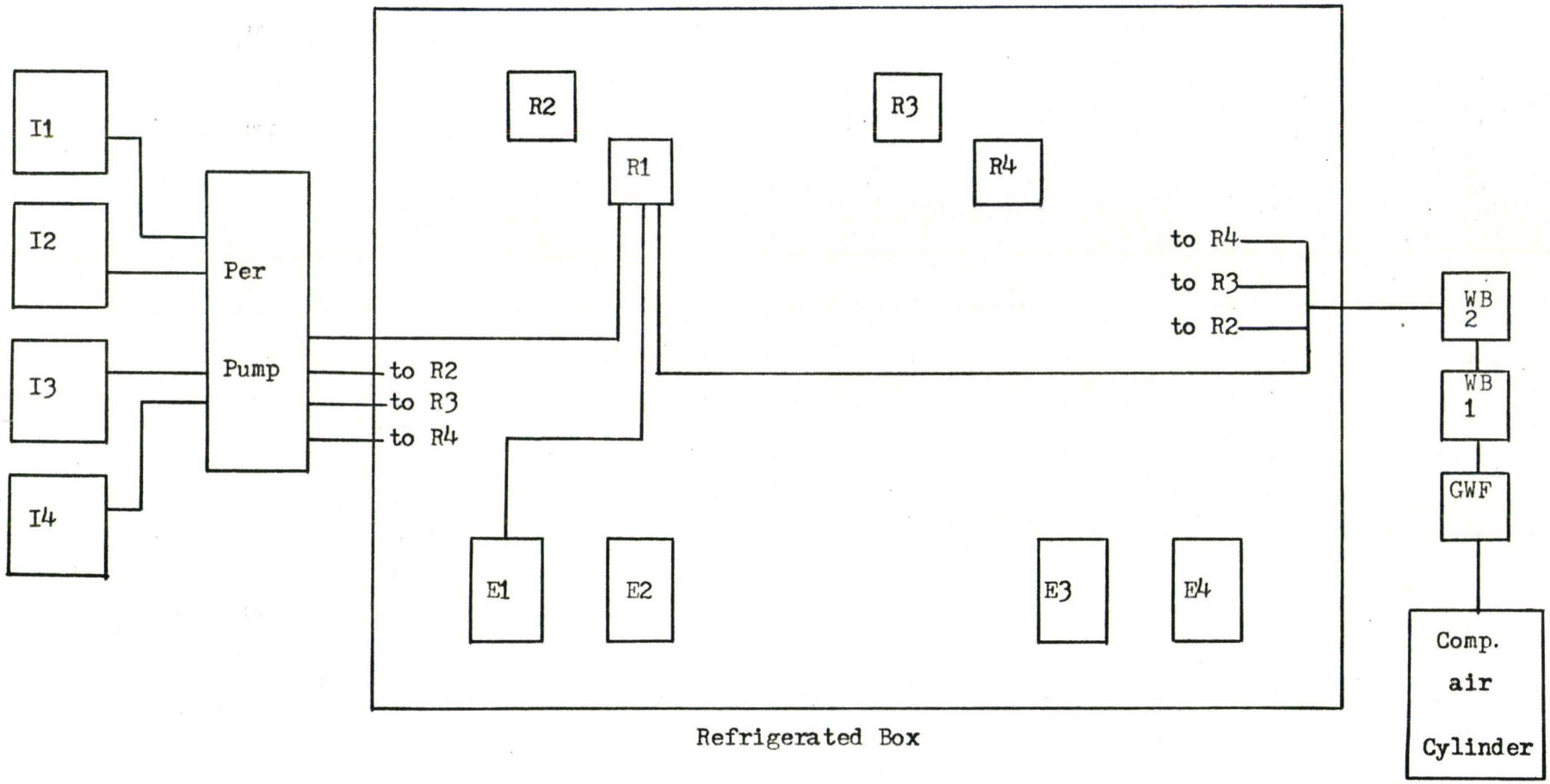
Light was provided by two 150 watt General Electric spotlights. These were chosen over fluorescent fixtures so that the voltage could be lowered without causing malfunctioning of the bulbs. The intensity of light was regulated by a potentiometer. Reciprocating motion of an electric motor, that was geared to turn two revolutions per day, was converted by means of a connecting rod to linear motion, which was used to control the

Figure 7




Chemostat Reactor Design

Figure 8




Schematic Diagram of Chemostat Reactor Including Influent, Effluent, and Air Supply

Figure 8

I1 : Influent #1
I2 : Influent #2
I3 : Influent #3
I4 : Influent #4

R1 : Reactor #1
R2 : Reactor #2
R3 : Reactor #3
R4 : Reactor #4

E1 : Effluent #1
E2 : Effluent #2
E3 : Effluent #3
E4 : Effluent #4

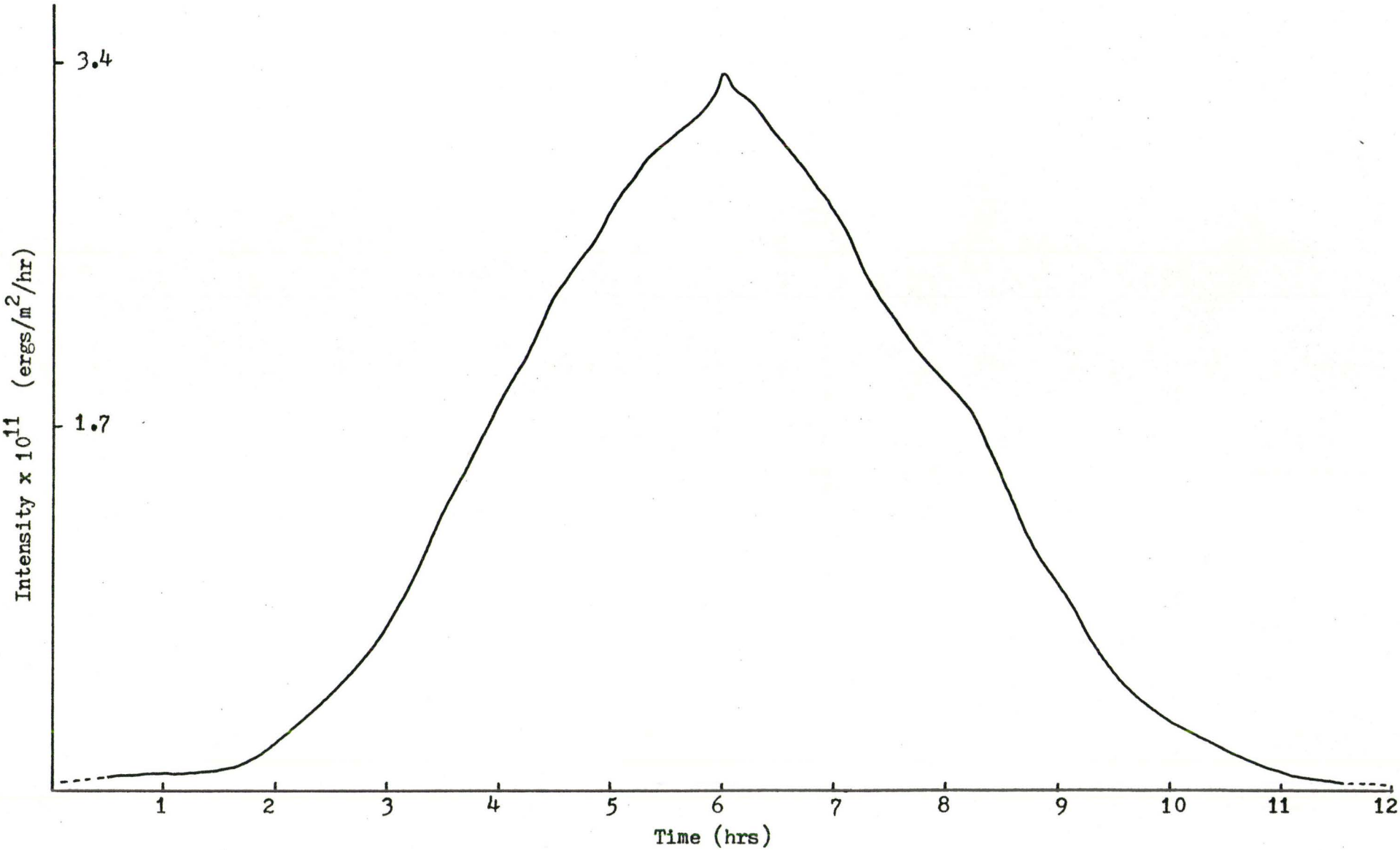
WB1 : Water Bath #1
WB2 : Water Bath #2

Per Pump : Peristaltic Pump

GWF : Glass Wool Filter

Figure 9

Light Intensity Incident on the Surface of the Culture Media Over a 12 Hour Period



potentiometer. The lights were switched on for 12 hours each day. The resulting light intensity is shown in figure 9 and the spectral curves are shown in figure 10. A C62 diamond blue filter was used to diffuse the light and attenuate the longer red wavelenghts.

Chemostat Housing:

The reactors were housed in an insulated plywood box (figure 11) containing one hundred feet of heat regulating copper tubing wound around its inside perimeter. The temperature of the box was controlled by pumping temperature regulated water through the coils at high speed by means of a Neslab refrigerated constant bath. An electric fan blowing across the reactors provide the needed air circulation. The top of the box consisted of a double paned glass window that allowed light into the unit while insulating the chemostat reactors from external heat fluxes.

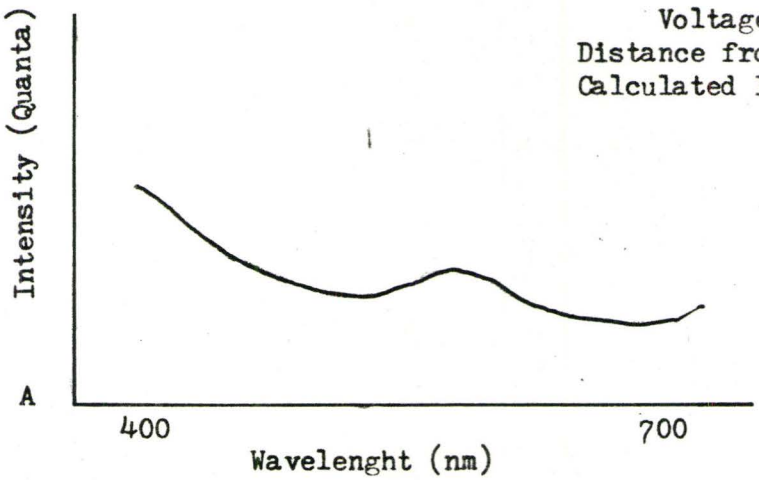
The inside of the refrigerated box was lined with aluminum foil to minimize the alteration of the spectral quality of the light as it reflected around the reactors. It had the additional affect of supplying reflected light from different angles within the cooler so that the algae were illuminated from more than one direction.

The light source was situated over the refrigerated box. It was housed in a light excluding box while an electric fan exhaused the excess heat that was generated by the bulbs.

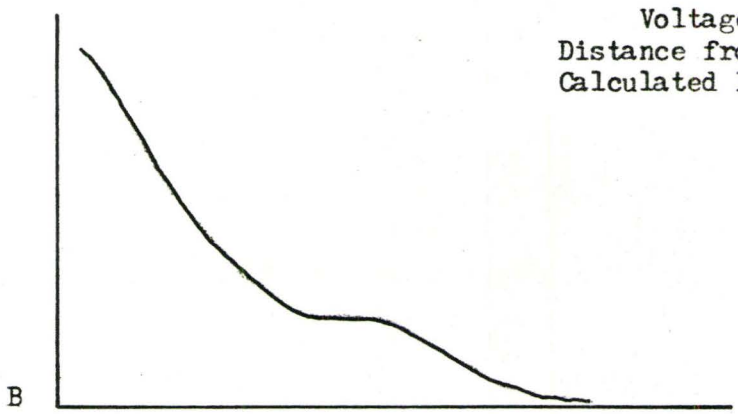
Culture Media:

Shales, limestones, dolomites and chert from the Niagara escarpment, fossiliferous limestone from the Trenton Ontario formation and minor amounts of pyrite from the Canadian shield

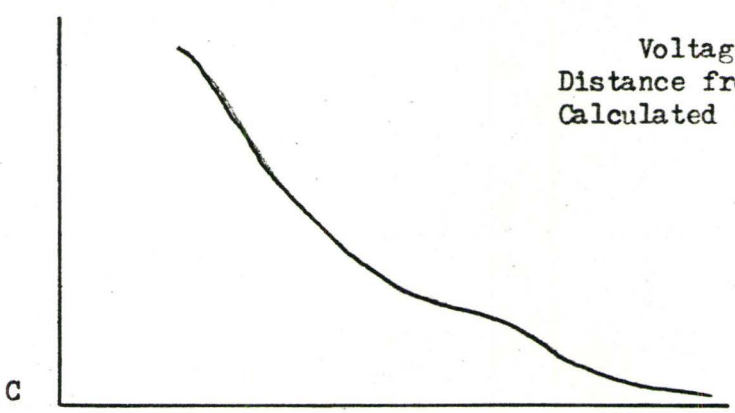
Figure 10




Voltage : 32.4
Distance from Spectrometer : 50cm
Calculated Intensity : $2.2085 \times 10^{9}$ ergs/$m^2$/hr




Voltage : 64.7
Distance from Spectrometer : 25cm
Calculated Intensity : $6.6925 \times 10^{11}$ ergs/$m^2$/hr




Voltage : 84.1
Distance from Spectrometer : 50cm
Calculated Intensity : $7.155 \times 10^{11}$ ergs/$m^2$/hr

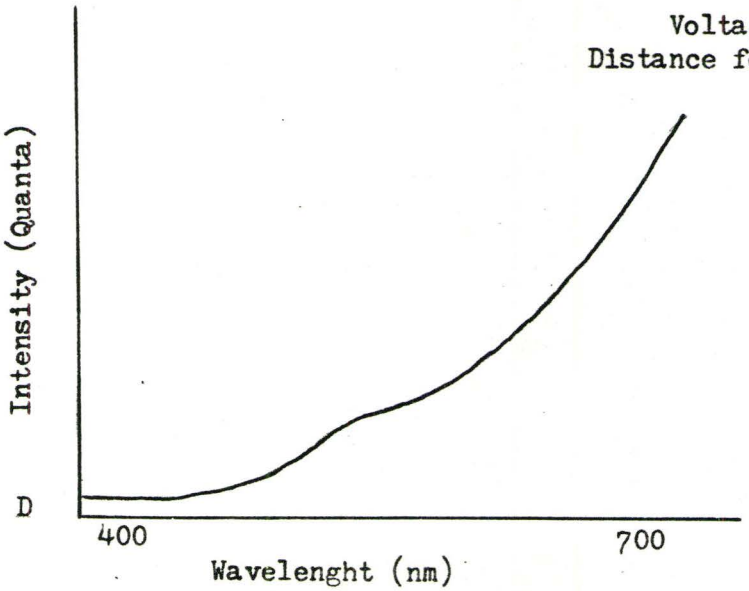
## Figure 10

Figures 10A-D show the spectral characteristics from 400 to 700 nm. from one G. E. spotlight at various voltages. The intensity of the red end of the spectrum decreases with respect to the rest of the curve as the voltage decreases. A secondary peak occurs at approximately 500 nm. when the voltage is only 32.4 .

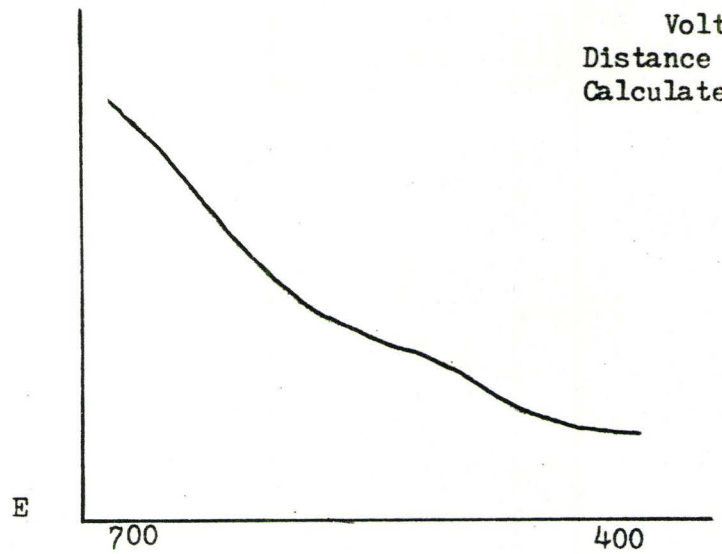
Calibration of a G. E. exposure meter (model 8DW58Y4) to the intensities calculated from the spectral curves at different voltages shows that a reading of 1 foot candle with the lid closed is equivalent to $1.13 \times 10^{10}$ ergs/$m^2$/hr.

Figures 10D and E show the spectral curves for one G. E. spotlight at the same voltage; D was not transmitted through glass while E was. The shape of the curves is approximately the same but the glass attenuates the longer (red) wavelengths.

Figure 10



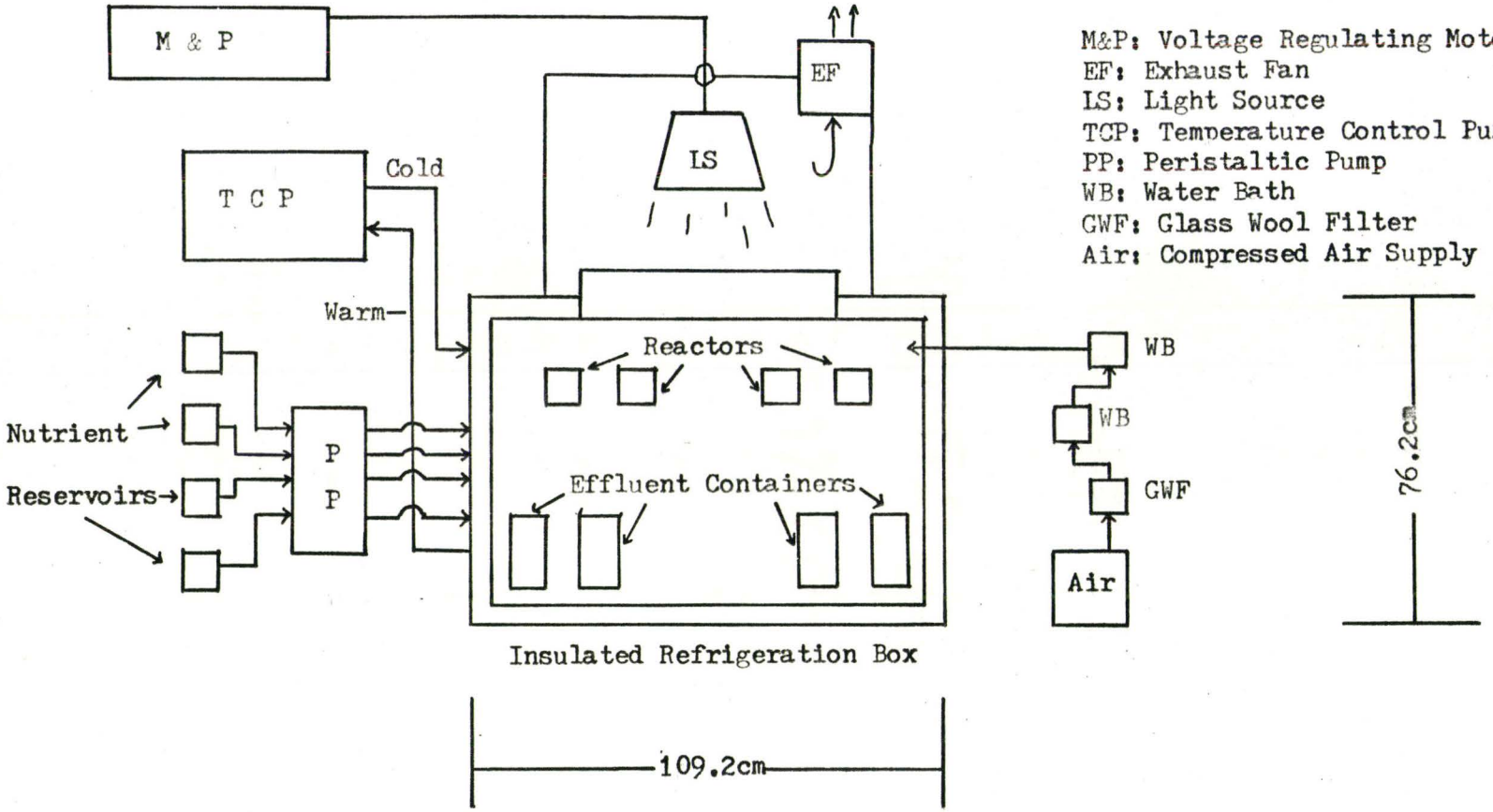

Voltage : 110
Distance form Spectrometer : 50cm




Voltage : 110
Distance from Spectrometer : 50cm
Calculated Intensity : $18.5087 \times 10^{11}$ ergs/$m^2$/hr

Figure 11

Schematic Chemostat Design



were crushed and stirred for one week in 40 litres of distilled water. 20 litres were centrifuged and filtered through a .45u millipore filter before sterilized and stored in 4 litre stoppered flasks. Care was always taken to prevent foreign algal spores from dropping into the flasks from the atmosphere. The following table (12) is a partial analysis of the components in the water.

Table 12

alkalinity: 1.0 meq $HCO_3$/l.

pH: 8.4

K: 1.08ppm

Na: .76ppm

N: not detected

P: not detected

Cu: 2ppb

Fe: 14ppb

Zn: 8.45ppb

Nitrate and phosphate were added as N and P by appropriate proportioning of 1000ppm N and P stock solutions made from $KNO_3$ and $K_2HPO_4$ respectively.

Algae:

Chlamydomonus rugosa was supplied by Dr. W. Glooshenko of C.C.I.W. His cultures were grown in the nutrient medium shown in the appendix. The culture consisted of one algal strain and was not bacteria free. A brief discussion of Chlamydomonus and its life cycle is given by Robbins et al (1957).

Procdure

1. All parts of the apparatus that would come in contact with the algae (either directly or indirectly) were disassembled and autoclaved where possible at 15psi. for 10 minutes. The 3 litre nutrient reservoirs were sterilized twice with boiling water. All bulk culture solutions were sterilized before using.

2. The stock algae cultures from C.C.I.W. were "preconditioned" in the nutrient solutions shown in table 13. Room temperature and 650 foot candles of light were used to promote growth. The lights were on for approximately 12 hours per day.

3. Four reactors were run at once. For each set of runs a constant phosphorus concentration was used while the nitrogen concentration was varied. Table 14 details the concentration of each set of runs.

4. A known population of Chlamydomonus rugosa was injected into the reactor. At all times the nutrient solution was kept isolated from the atmosphere and sterile pipettes were used to transfer the inoculum.

5. Cell counts were made (see appendix 2) every 3 or 4 days. Since growth depends on the lighting of the algae population, estimates of the number of cells must be made at the same time on each sample day. Once the population of cells has remained constant for one hydraulic residence the nitrogen and phosphorus can be analysed.

6. Phosphate and nitrate concentrations were determined by colourimetric techniques using standard stannous chloride and cadmium reduction procedures respectively.

## Results

The influent and effluent analyses are given in table 15. The cell counts are given in table 16. The flow rates of the systems were $7.0 \pm 0.5$ ml./hr.

The N and P analyses show that neither was depleted significantly during the course of the experiment. The slight decrease in the nutrient concentrations in the reactor may be due to algal uptake, adsorption onto the chemostat wall and errors in analyzing. Population estimates reveal that the cells increased in number when they were first introduced into the chemostats but no division took place after day two. Microscope examinations reveal that few cells entered the mitotic stage of reproduction after day 4. The cells in reactor 1 died and decomposed after day 27 but no anomolous results were recorded in the concentrations of N and P in the reactor.

Table 13

Preconditioned Samples

1. 60 ppb $PO_4$/P
   20 ppb $NO_3$/N

2. 30 ppb $PO_4$/P
   100 ppb $NO_3$/N

3. 100 ppb $PO_4$/P
   200 ppb $NO_3$/N

4. 60 ppb $PO_4$/P
   100 ppb $NO_3$/N

5. 60 ppb $PO_4$/P + 1ml L. Ont. water per 150 ml of culture sol$^n$
   100 ppb $NO_3$/N

6. 100 ppb $PO_4$/P
   200 ppb $NO_3$/N

7. 1 ppm $NO_3$/N
   100 ppb $PO_4$/P

8. 20 ppb $PO_4$/P
   29 ppm $NO_3$/N

9. 100 ppb $PO_4$/P + 1ml L. Ont water per 150 ml of culture sol$^n$
   1 ppm $NO_3$/N

Table 14

| Set | Nutrient ppb | Flask 1 | Flask 2 | Flask 3 | Flask 4 |
|---|---|---|---|---|---|
| 1 | $NO_3$/N | 10 | 100 | 500 | 2000 |
| | $PO_4$/P | 30 | 30 | 30 | 30 |
| 2 | $NO_3$/N | 10 | 100 | 500 | 2000 |
| | $PO_4$/P | 100 | 100 | 100 | 100 |
| 3 | $NO_3$/N | 10 | 100 | 500 | 2000 |
| | $PO_4$/P | 300 | 300 | 300 | 300 |
| 4 | $NO_3$/N | 10 | 100 | 500 | 2000 |
| | $PO_4$/P | 1000 | 1000 | 1000 | 1000 |

Table 15

| | Reactor 1 | Reactor 2 | Reactor 3 | Reactor 4 |
|---|---|---|---|---|
| Influent P | 100 | 101 | 101 | 100 |
| Effluent P | 98 | 98 | 98 | 97 |
| Influent N | 10 | 100 | 495 | 1990 |
| Effluent P | 10 | 100 | 490 | 1980 |

Table 16

| | Initial Pop. | 2 Days | 4 Days | 7 Days | 10 Days | 15 Days | 21 Days | 25 Days | 27 Days | 36 Days |
|---|---|---|---|---|---|---|---|---|---|---|
| Reactor 1 | 67/ml | 90 | 105 | 100 | 100 | 95 | 100 | 95 | 95 | 0 |
| Reactor 2 | 67/ml | 90 | 105 | 100 | 100 | 95 | 100 | 100 | 100 | 95 |
| Reactor 3 | 67/ml | 90 | 105 | 100 | 100 | 95 | 100 | 100 | 100 | 100 |
| Reactor 4 | 67/ml | 95 | 110 | 100 | 105 | 95 | 100 | 105 | 100 | 105 |

## Discussion

The N and P analyses show that neither nutrient was growth limiting. The light intensities and reactor temperatures were sufficient to promote algal growth. Random analyses of trace metals showed detectable concentrations in solution. Organic molecules were added to the culture media from the weathered rocks that were used to supply cations to the nutrient solution. Bacterial decay of non viable algae supplemented the supply of organic molecules in the culture. Therefore it appears that growth of the algae was not limited by the absence of organic molecules. Even the preliminary samples that contained Lake Ontario water (containing organic molecules) did not result in algal growth.

The only preliminary batch cultures that bloomed occurred in the medium that was greatly enriched in the nutrients (ie. 20ppm $PO_4$/P and 29ppm $NO_3$/N)

The possibility of toxic material inhibiting the growth of the algae is refuted by the bloom that occurred in the high nutrient concentrations even though the Cu concentration, at 2ppb, is high. Perhaps the high N and P concentrations offset any limiting substrate that prevents growth when N and P are found at lower abundances.

Comparison (table 17) of the medium that the stock cultures grew in and the medium of the experiment show large discrepancies in the abundances of the constituents.

The variation between the media might prevent logarithmic growth in the experimental samples at the lower substrate concen-

trations. Some algae, under unfavourable conditions, can remain in the "resting" stage of growth for months (E. Mills personal communication). Rapid growth may also be hindered by the lack of red light and the discontinuous nature of the light intensity that was used in this experiment.

Table 17

| | CCIW Medium | Experimental media Growth | No Growth |
|---|---|---|---|
| N | 4.2ppm | 29ppm | 2ppm |
| P | .186ppm | 21ppm | .1ppm |
| K | .469ppm | 1.08ppm | 1.08ppm |
| Na | 11.00ppm | .76ppm | .76ppm |
| Fe | 33ppb | 14ppb | 14ppb |
| Zn | 15.7ppb | 8.45ppb | 8.45ppb |
| Cu | .004ppb | 2ppb | 2ppb |

Chemostat Design

For convenience, the chemostat reactors should not be inverted. Reactors that are accessible from the top allow easy input of nutrients, and inoculation of algae. Additionally air baths are only borderline efficient when high heat producing light sources are used. Water baths should be used when high heat fluxes are expected.

## APPENDIX 1

## CCIW Culture Media

### Macronutrients

| Compound | Concentration (mg/l) | Element | Concentration (mg/l) |
|---|---|---|---|
| $NaNO_3$ | 25.500 | N | 4.200 |
| $K_2HPO_4$ | 1.044 | P | 0.186 |
| $MgCl_2$ | 5.700 | Mg | 2.904 |
| $MgSO_4 \cdot 7H_2O$ | 14.700 | S | 1.911 |
| $CaCl_2 \cdot 2H_2O$ | 4.410 | C | 2.143 |
| $NaHCO_3$ | 15.000 | Ca | 1.202 |
| | | Na | 11.001 |
| | | K | 0.469 |

### Micronutrients

| Compound | Concentration (ug/l) | Element | Concentration (ug/l) |
|---|---|---|---|
| $H_3BO_3$ | 185.520 | B | 32.460 |
| $MnCl_2$ | 264.264 | Mn | 115.374 |
| $ZnCl_2$ | 32.709 | Zn | 15.691 |
| $CoCl_2$ | 0.780 | Co | 0.354 |
| $CuCl_2$ | 0.009 | Cu | 0.004 |
| $Na_2MoO_4 \cdot 2H_2O$ | 7.260 | Mo | 2.878 |
| $FeCl_3$ | 96.000 | Fe | 33.051 |
| $Na_2EDTA \cdot 2H_2O$ | 300.000 | | |

## APPENDIX 2

### Cell Counting

1. The volume of one drop of culture solution was measured.

2. One drop of culture solution was evaporated to dryness on a glass plate and its area measured.

3. The number of organisms were counted per unit area.

4. These value were extrapolated to populations per ml.

5. Several counts were taken and the averages recorded.

6. If cell populations were too high the samples could be diluted by a known volume of water. When extrapolating the dilution was also used.

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