SERI/CP-624-1228

Proceedings of the Subcontractors' Review Meeting Aquatic Species Program

Sponsored and Organized by:

Solar Energy Research Institute
Biomass Program Office

for the

U.S. Department of Energy

July 1, 1981
Washington, D.C.

NOTICE

SERI/CP-624-1228

PROCEEDINGS OF THE
SUBCONTRACTORS' REVIEW MEETING
AQUATIC SPECIES PROGRAM

JULY 1, 1981

WASHINGTON, D. C.

SPONSORED AND ORGANIZED BY:

SOLAR ENERGY RESEARCH INSTITUTE
BIOMASS PROGRAM OFFICE

FOR THE

U.S. DEPARTMENT OF ENERGY

TABLE OF CONTENTS

YEAR ONE PROGRESS REPORT ON ALGAL PRODUCTION RACEWAY PROJECT

E.A. Laws
University of Hawaii
Oceanography Dept.
2525 Correa Rd.
Honolulu, HI 96822

INTRODUCTION

The principal objectives of the Algal Production Raceway (APR) project are twofold. First, we want to confirm that lipid and protein production can be maintained over a six-month period at a sufficiently high rate to make the project economically and energetically attractive. Second, we want to develop a harvesting mechanism which can remove cells from the APR at a satisfactory rate and with a minimum of energy expendature.

Much of the culture work during the first year of the project has involved small volume chemostats, since construction of the raceway systems was not completed until January, 1981. The chemostat work has allowed us to thoroughly examine the growth and compositional characteristics of Phaeodactylum tricornutum as a function of light and nutrient limitation, over a range of temperatures and as a function of light quality. These studies clearly showed that maximum lipid and protein production would be achieved under light-limited conditions, and near the end of the first year a thorough study of protein and lipid production under light-limited conditions in two strains of P. tricornutum revealed a 40% and 70% difference in lipid and protein production respectively between the two strains. This difference in production is quite important to the overall economics of the project.

During the first few months of operation of the APR, we were able to explore several different cell harvesting mechanisms. At the moment the most promising method appears to be collection of foam, which contains 10-40 times the cell density in the APR and is produced in more-than-adequate amounts by aeration of the raceway when culture densities reach a few million cells per ml. The foaming is apparently the result of the release of surfactants by the cells. A compositional analysis of cells collected from the APR has revealed results very similar to those reported earlier for P. tricornutum in small-scale APR prototypes.

The chief disappointment in the initial work on the APR has been our failure to produce cell densities above about 5×10^6 cells$\cdot$ml^{-1} at growth rates comparable to those needed to make the APR concept economically attractive. Earlier results from the APR had indicated that growth rates of about 0.4 d^{-1} could be achieved at cell densities of about 2.5×10^7 cells$\cdot$ml^{-1}, but cell densities in our APR have never exceeded about 1.1×10^7 cells$\cdot$ml^{-1}, and growth rates at these high cell densities have been well below 0.4 d^{-1}. Even with cell densities of 2.5×10^7 cells$\cdot$ml^{-1}, our calculations indicates that an APR would have to be about 0.6 m deep in order to achieve maximum production. The system was initially envisioned as being operated with a depth of only a few cm and at a very high cell density, but it appears that release of inhibatory metabolites by the cells will prevent us from operating the system in that mode. We have therefore redesigned the APR to accommodate a greater culture depth.

CARBON PRODUCTION BY P. TRICORNUTUM BASED ON CONTINUOUS CULTURE RESULTS

Consider an APR system having a depth D meters in which P. tricornutum is growing at a rate μ per day. If the concentration of particulate carbon in the raceway is C g$\cdot$m^{-3}, then the rate of production of carbon is μCD g$\cdot$m^{-2}d^{-1}. If

C' is the concentration of Chl *a* in the raceway, then attenuation of light with depth in the culture can be well described by the equation

$$I_Z = I_o e^{-KC'Z} \quad (1)$$

where I_Z is the light intensity at depth Z, Io is the surface light intensity, and K is the extinction coefficient of light per unit Chl *a*. The average light intensity I in the water column is then

$$I = \frac{1}{D}\int_0^D I_Z dZ = \frac{Io}{KC'D}\,(1 - e^{-KC'D}) \quad (2)$$

For reasons which will soon become clear, we can assume the APR will be run under conditions in which $e^{-KC'D} \ll 1$, so that

$$I \cong \frac{Io}{KC'D} \quad (3)$$

Hence production in the raceway can be written as

$$\mu CD = \frac{\mu C}{C'} \cdot C'D = \frac{\mu CIo}{C'KI} \quad (4)$$

Most values reported for K fall in the range 10 to 20 $m^2 \cdot g^{-1}$ Chl *a*. In the actual APR however, we can expect that substances other than Chl *a* will scatter and/or absorb light. Experiments conducted by us indicate a K value of about 23 at high cell densities, and measurements made in the APR in early May, 1981, yielded a K of 22. In modeling production, we have assumed a K value of 22 $m^2 \cdot g^{-1}$ Chl *a*.

Average incident solar radiation in Hawaii is estimated to be about 3.1×10^{10} $BTU \cdot acre^{-1} \cdot yr^{-1}$. Taking 50% of this figure to be visible light (400 - 700 nm), assuming a 12h photoperiod, and using appropriate conversion factors, we calculate the mean incident light intensity to be about 1200 $\mu Ein \cdot m^{-2} \cdot s^{-1}$. Mean light intensities recorded by us at the APR site during May, 1981, were 1150 $\mu Ein \cdot m^{-2} \cdot s^{-1}$. We have assumed an Io of 1200 in the following calculations.

1. Light-Limited Carbon Production

Figures 1 and 2 show plots of growth rate and the carbon:Chl *a* ratio (C:C') as a function of I for the Woods Hole (WH) strain of *P. tricornutum*. Analogous data have been obtained for the West Coast (Thomas) strain. Using the smooth curves fit to these data, the plots of total carbon production as a function of I were generated in Figs. 3 and 4. For both strains, carbon production peaked at an I of about 35 $\mu Ein \cdot m^{-2} \cdot s^{-1}$, but production was about 63% higher in the Thomas strain. The principal difference in the two strains was in the C:C' ratio, which was much higher in the Thomas strain. Assuming that carbon accounts for about 55% of the ash free dry weight (AFDW) of *P. tricornutum*, these production estimates translate into 35.9 and 58.3 short tons AFDW per acre-year. The latter figure compares favorably with the estimate of 62.5 short tons AFDW per acre-year in the original APR proposal.

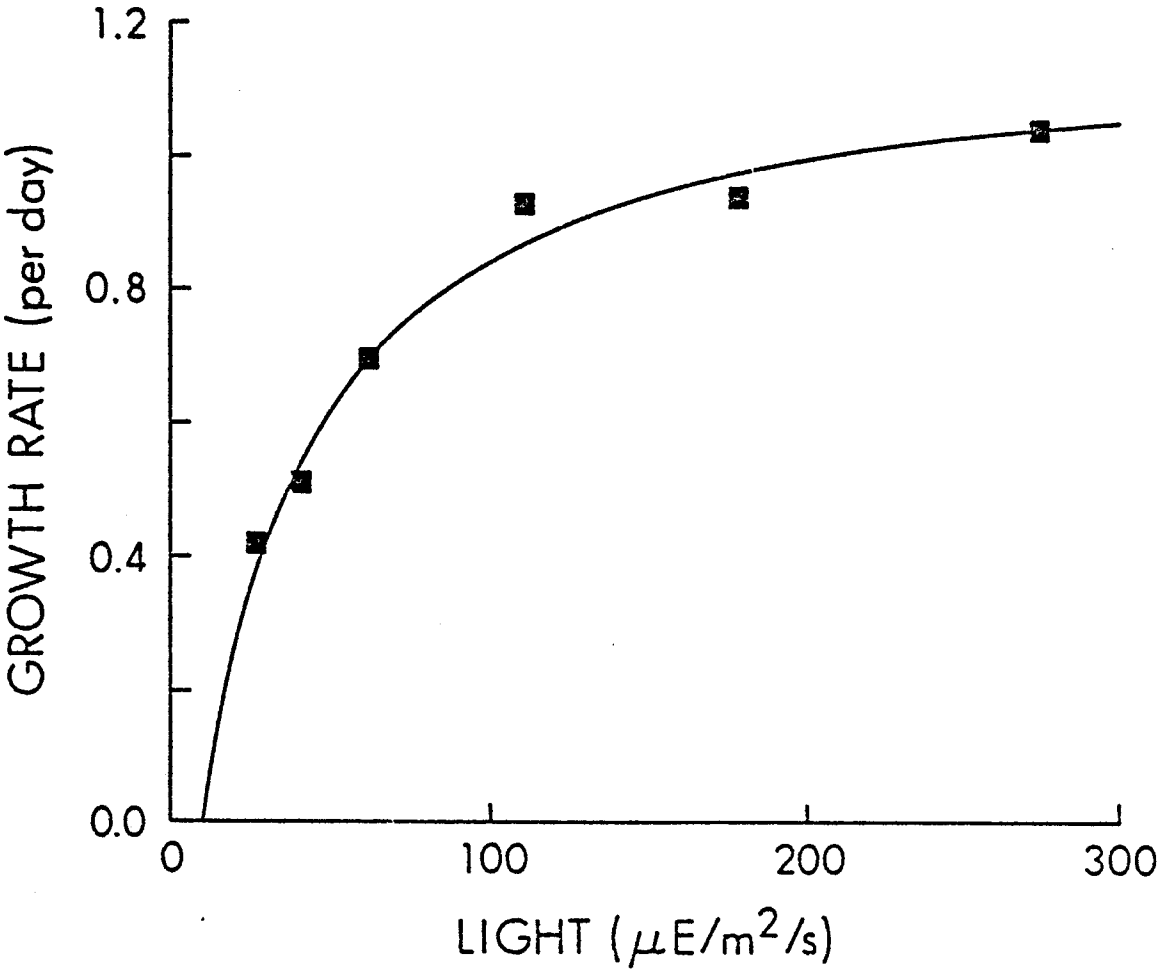


FIGURE 1. GROWTH RATE OF THE WOODS HOLE STRAIN OF PHAEODACTYLUM TRICORNUTUM AS A FUNCTION OF VISIBLE LIGHT INTENSITY WHEN GROWN ON A 12:12 L:D CYCLE.

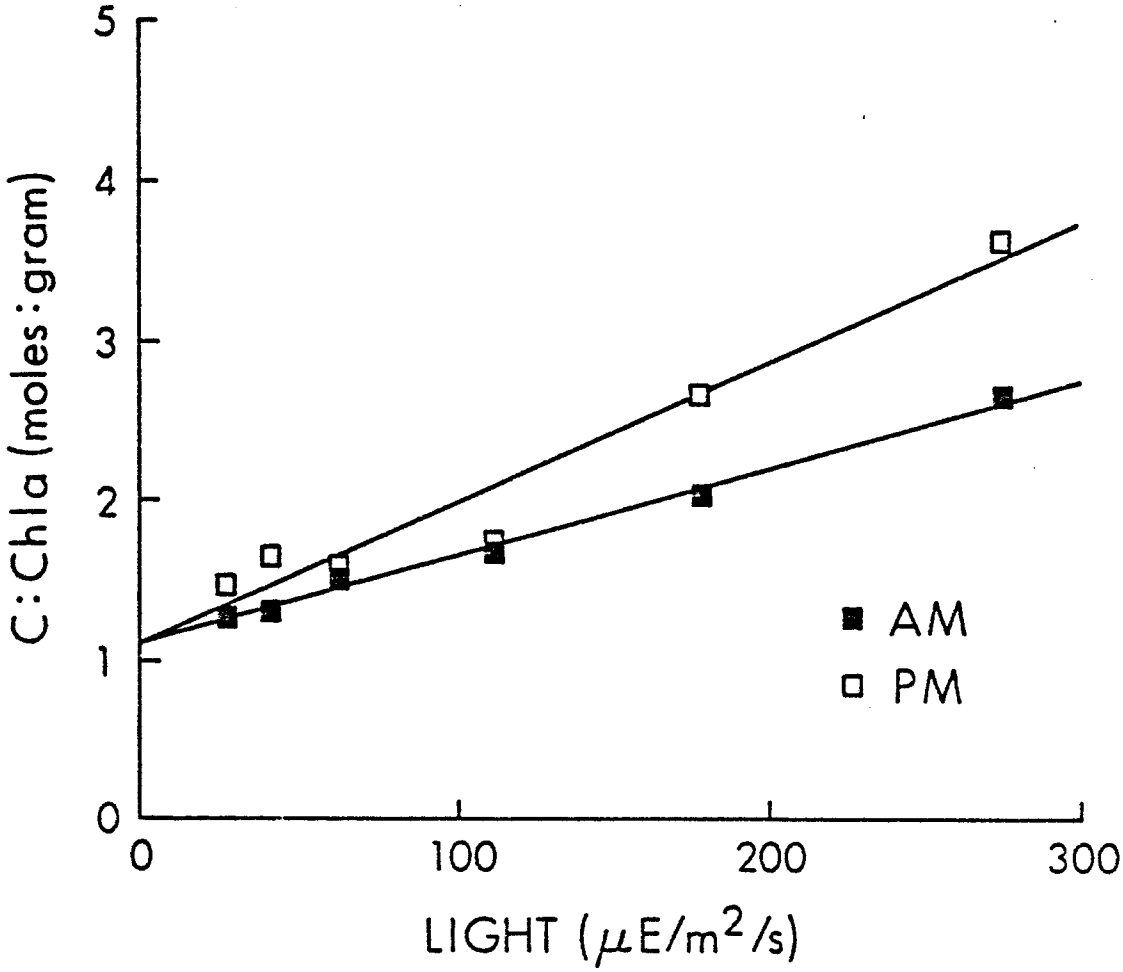


FIGURE 2. THE CARBON TO CHLOROPHYLL A RATIO IN THE WOODS HOLE STRAIN AS A FUNCTION OF LIGHT INTENSITY UNDER THE SAME CONDITIONS AS IN FIGURE 1. AM AND PM SAMPLES WERE COLLECTED AT LIGHTS ON AND LIGHTS OFF RESPECTIVELY.

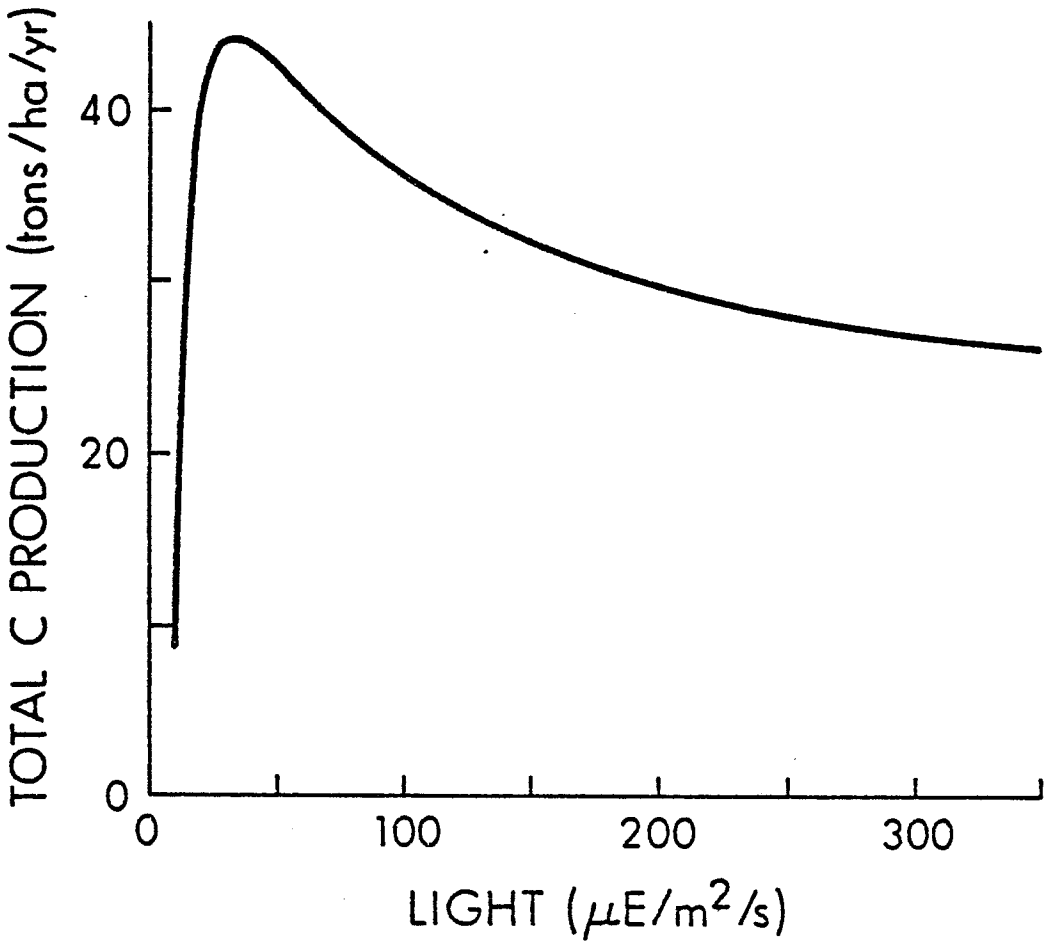


FIGURE 3. TOTAL CARBON PRODUCTION AS A FUNCTION OF LIGHT INTENSITY FOR THE WOODS HOLE STRAIN USING EQUATION 4 AND THE CURVES IN FIGURES 1 AND 2.

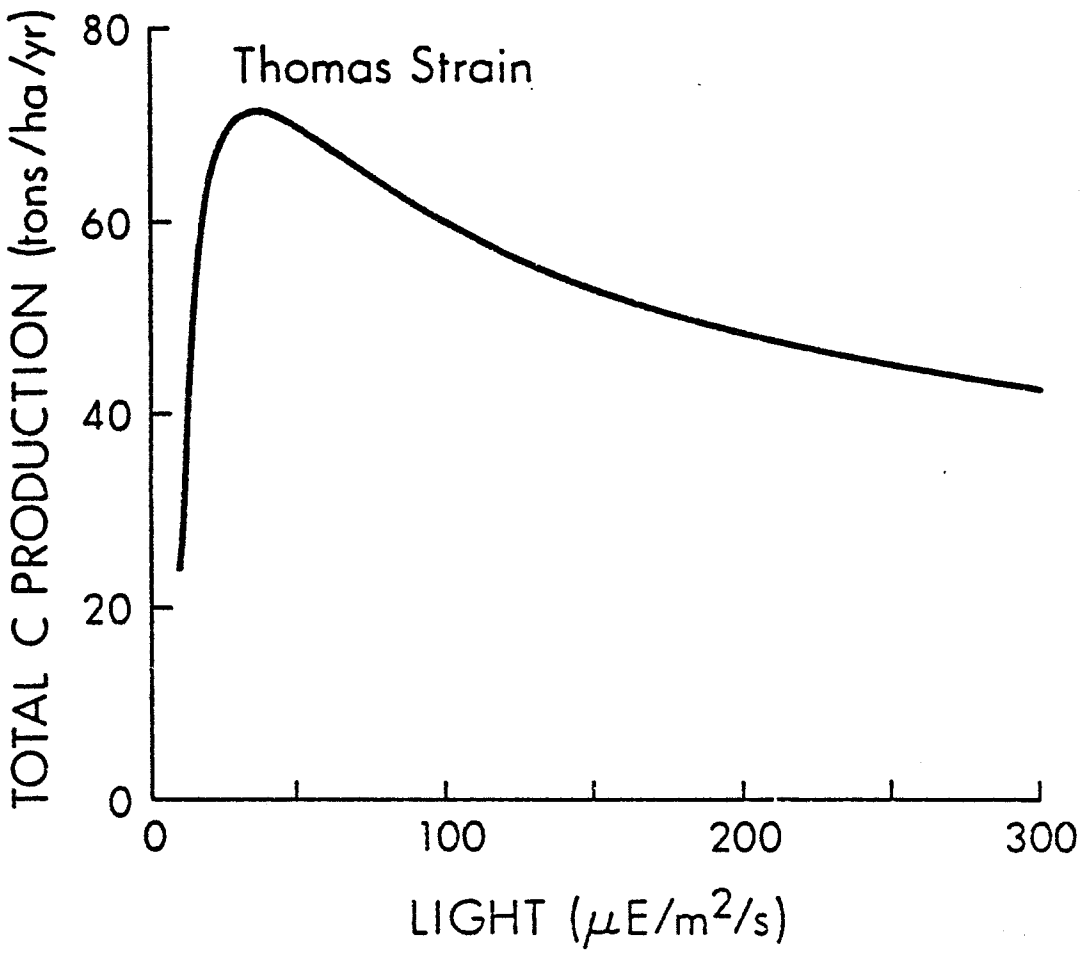


FIGURE 4. TOTAL CARBON PRODUCTION AS A FUNCTION OF LIGHT INTENSITY FOR THE THOMAS STRAIN USING EQUATION 4 AND DATA ANALOGOUS TO THAT IN FIGURES 1 AND 2.

2. Light-Limited Lipid Production

Figure 5 shows the percentage of cellular carbon as lipid as a function of I for the WH strain. An analogous plot was obtained for the Thomas strain. Lipid carbon production can be calculated as a function of I by multiplying the right-hand side of eq. 4 by the fraction of cellular carbon in lipid. The results for the WH and Thomas strains are shown in Fig. 6 and 7. Peak lipid C production for the WH and Thomas strains is estimated to be 15.8 and 22.5 tons $C \cdot ha^{-1} \cdot yr^{-1}$ at a light intensity of 35 $\mu Ein \cdot m^{-2} \cdot s^{-1}$. Assuming that carbon accounts for about 74% of the AFDW of the lipid, these figures translate into 9.5 and 13.5 short tons lipid AFDW per acre-year respectively. Assuming that this lipid consists of 70% free oils with an energy content of 17,000 BTU per pound, and that a barrel of oil is equivalent to 6×10^6 BTU, the free oil production rate is calculated to be 37.7 and 53.7 bbl per acre-year respectively.

3. Light-Limited Protein Production

Figure 8 shows the percentage of cellular carbon as protein as a function of I for the WH strain. An analogous plot was obtained for the Thomas strain. Multiplying the right-hand side of eq. 4 by the fraction of cellular carbon as protein yields the graphs of protein production shown in Figs. 9 and 10. Maximum production again occurred at a light level of 35 $\mu Ein \cdot m^{-2} \cdot s^{-1}$, and equaled 18.6 and 31.7 tons $C \cdot ha^{-1} \cdot yr^{-1}$ for the WH and Thomas strains respectively. Assuming carbon to account for 51.3% of protein AFDW, these numbers translate into 16.2 and 27.6 short tons protein AFDW per acre-year.

4. Effect of Light on Production at a Fixed Nutrient-Limited Growth Rate

We consider here only the growth rate 0.41 d^{-1}. Although experiments were also performed at 0.75 d^{-1}, the results at this second growth rate are qualitatively similar to the results at 0.41 d^{-1}. The calculations were made using eq. 4 for total carbon production, and by multiplying eq. 4 by the fraction of cellular carbon as lipid or protein to determine lipid and protein production respectively. For the sake of brevity, we include only the final results here. These results are summarized in Figs. 11-13.

It is clear from these figures that reducing the light level in a nutrient-limited culture (by either increasing the culture density or increasing the depth of the culture), so as to ultimately produce a light-limited culture at the same growth rate increases the rate of production per unit area of the culture. If this conclusion is true at all growth rate, then maximum production is obviously obtained in a light-limited system. In order to check this hypothesis, we performed a series of studies under nutrient-limited conditions.

5. Effect of Nutrient Limitation on Production at I = 300 uEin $\cdot m^{-2} \cdot s^{-1}$

The results reported here were obtained in a manner similar to those previously

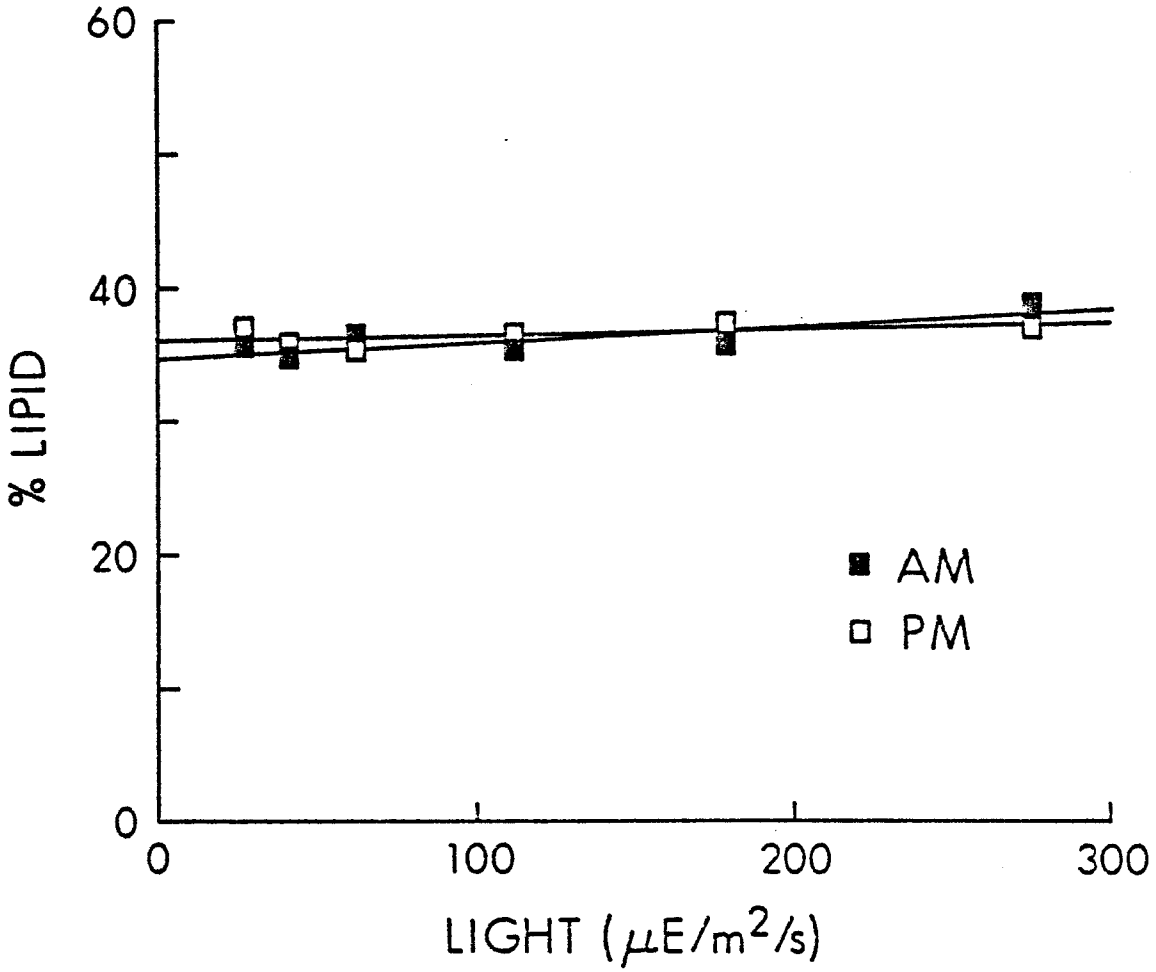


FIGURE 5. PERCENT OF CELLULAR CARBON AS LIPID CARBON IN THE WOODS HOLE STRAIN AS A FUNCTION OF LIGHT INTENSITY.

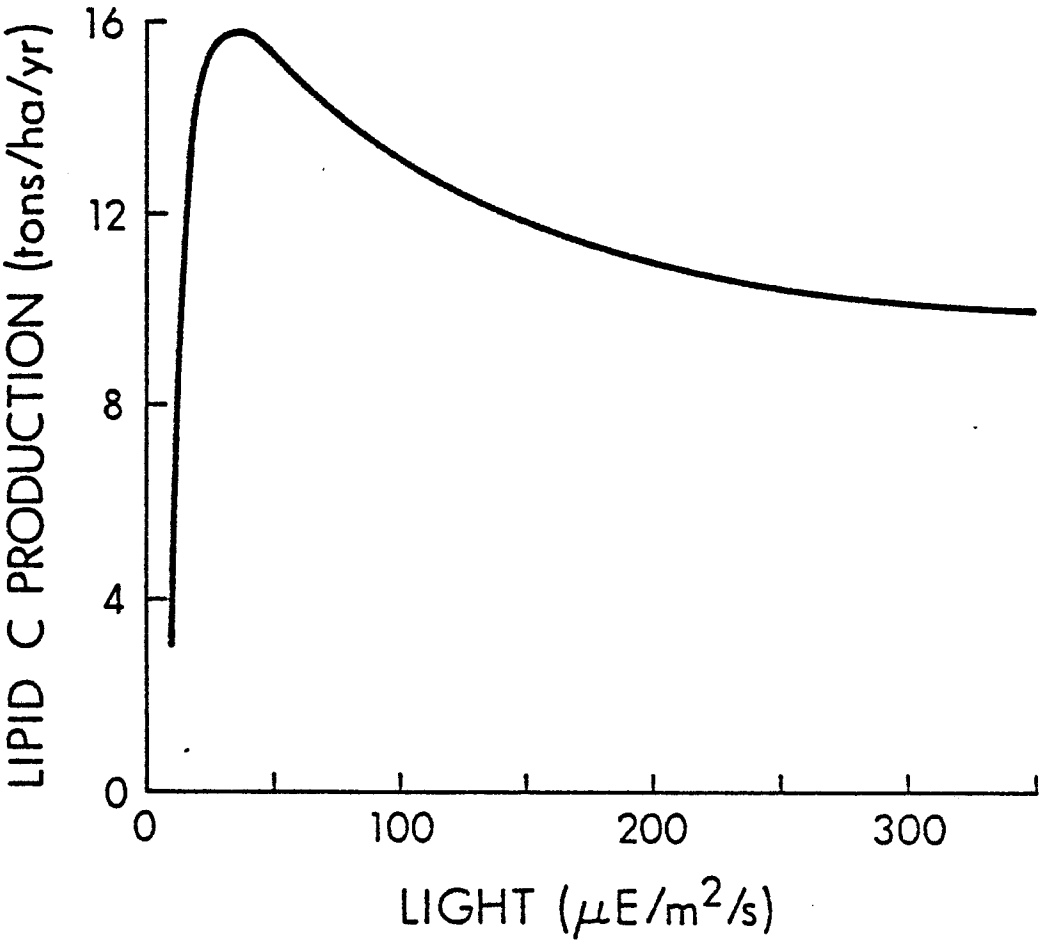


FIGURE 6. LIPID CARBON PRODUCTION AS A FUNCTION OF LIGHT INTENSITY IN THE WOODS HOLE STRAIN CALCULATED FROM THE PRODUCT OF THE CURVES IN FIGURES 3 AND 5.

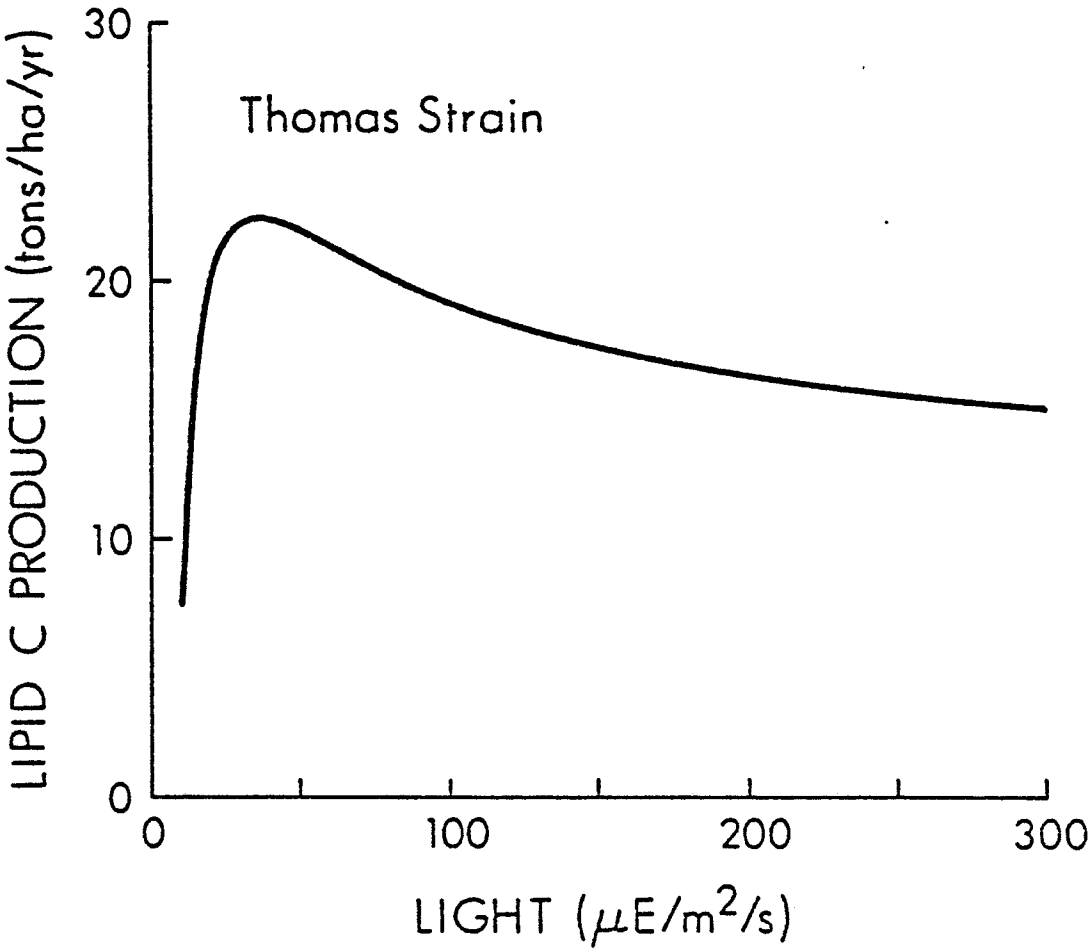


FIGURE 7. LIPID CARBON PRODUCTION FOR THE THOMAS STRAIN CALCULATED AS THE PRODUCT OF THE CURVE IN FIGURE 4 AND A PLOT ANALOGOUS TO FIGURE 5.

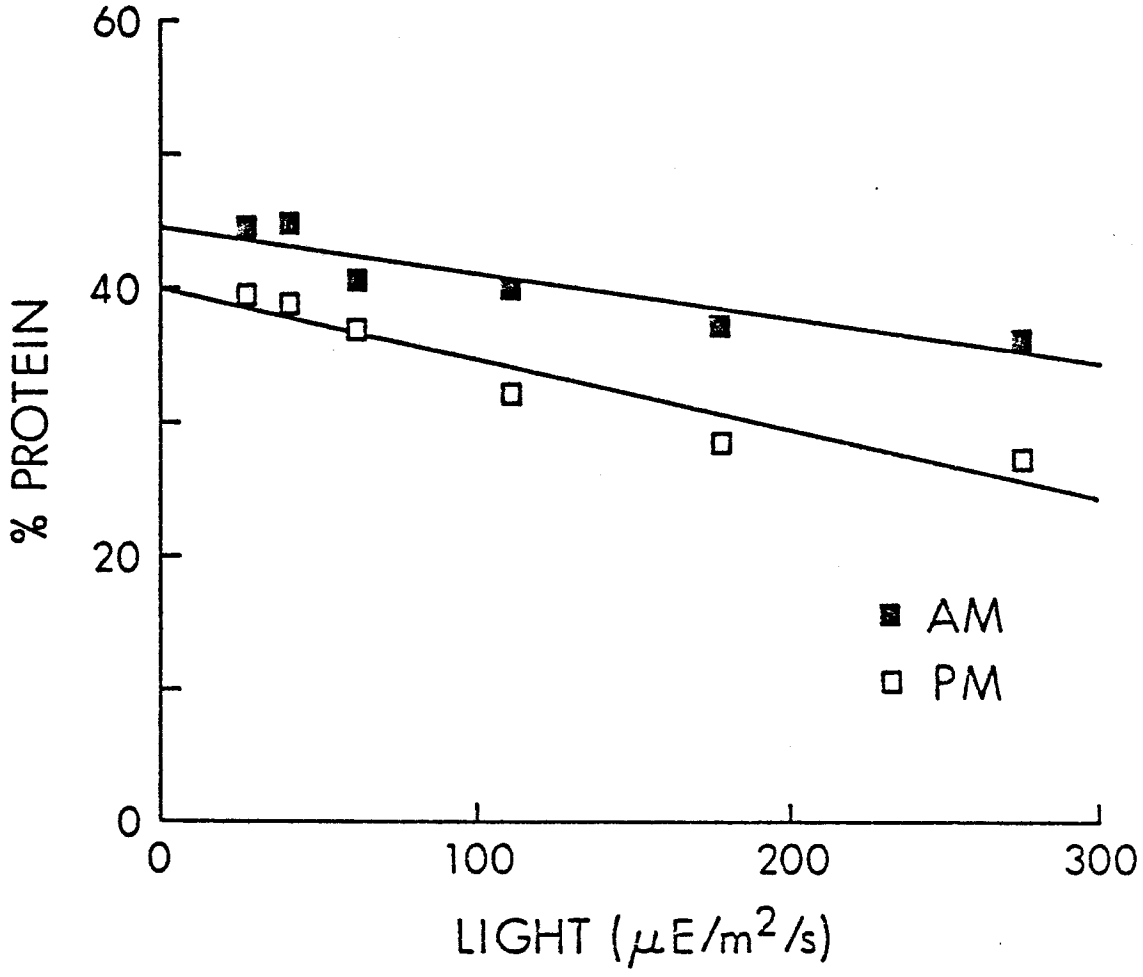


FIGURE 8. PERCENT OF CELLULAR CARBON AS PROTEIN CARBON IN THE WOODS HOLE STRAIN AS A FUNCTION OF LIGHT INTENSITY.

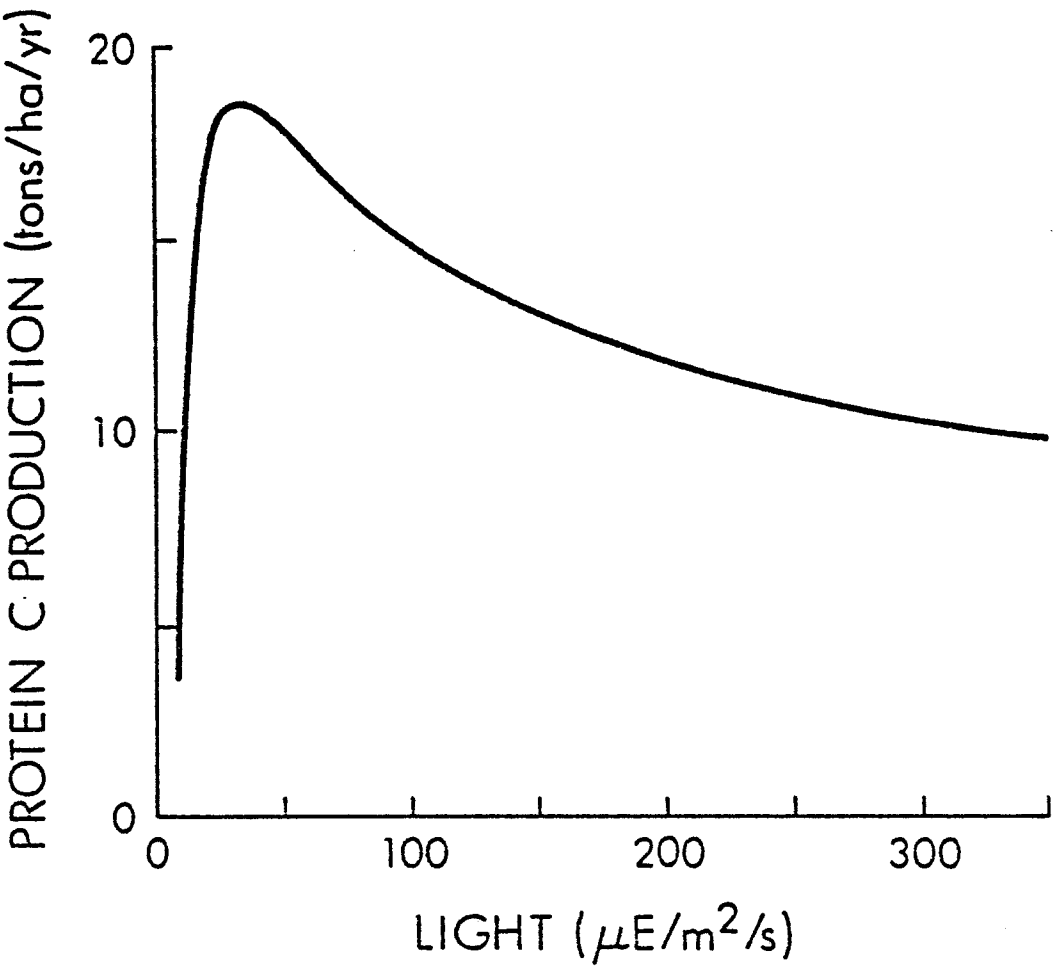


FIGURE 9. PROTEIN CARBON PRODUCTION AS A FUNCTION OF LIGHT INTENSITY IN THE WOODS HOLE STRAIN CALCULATED FROM THE PRODUCT OF THE CURVES IN FIGURES 3 AND 8.

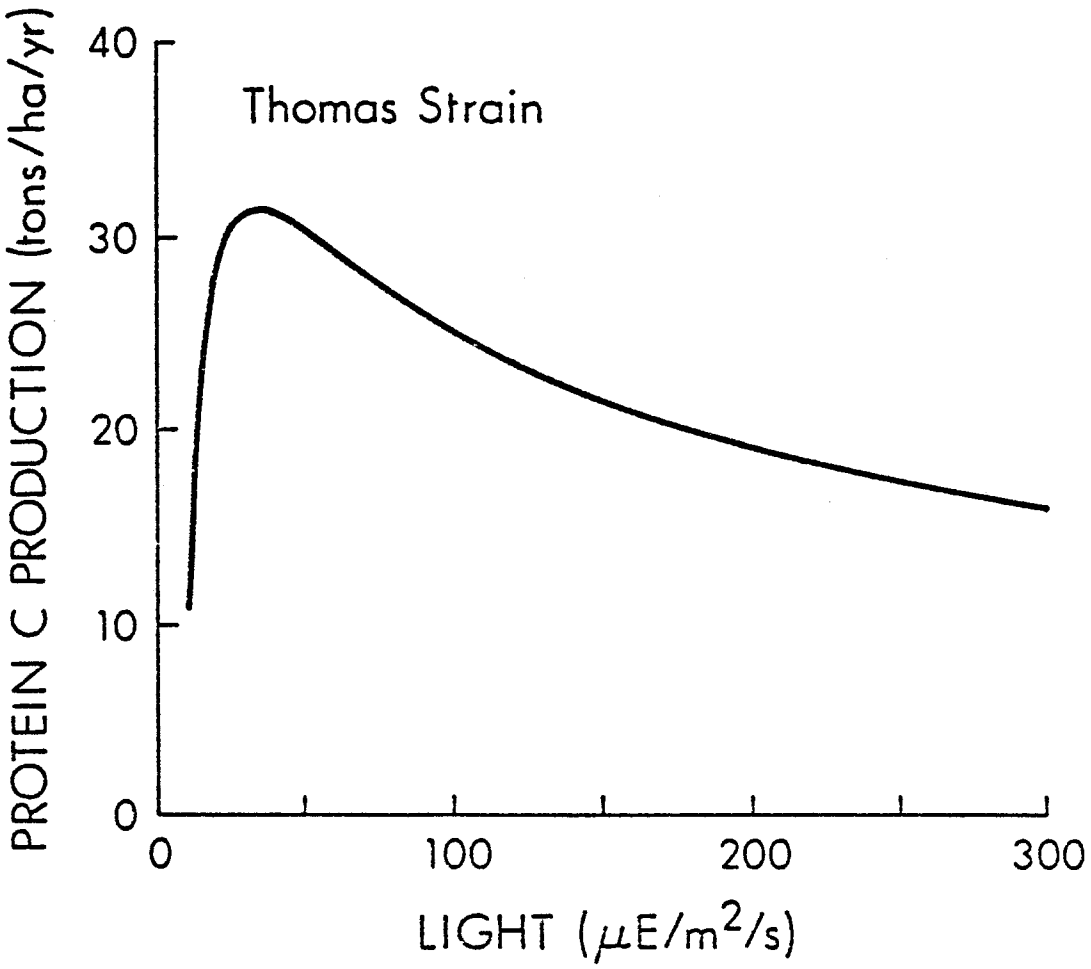


FIGURE 10. PROTEIN CARBON PRODUCTION AS A FUNCTION OF LIGHT INTENSITY IN THE THOMAS STRAIN CALCULATED AS THE PRODUCT OF THE CURVE IN FIGURE 4 AND A PLOT ANALOGOUS TO FIGURE 8.

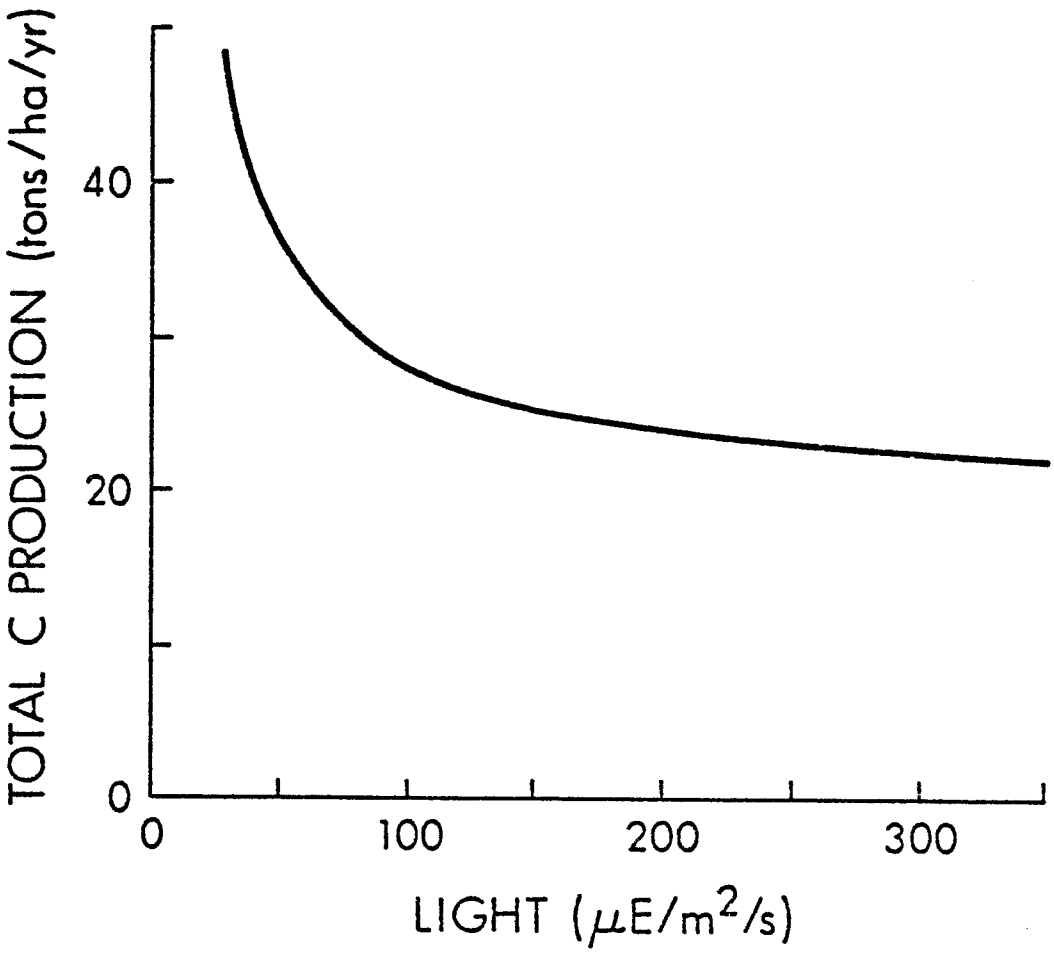


FIGURE 11. TOTAL CARBON PRODUCTION AS A FUNCTION OF LIGHT INTENSITY IN A NUTRIENT-LIMITED POPULATION OF THE WOODS HOLE STRAIN GROWING AT 0.41 PER DAY.

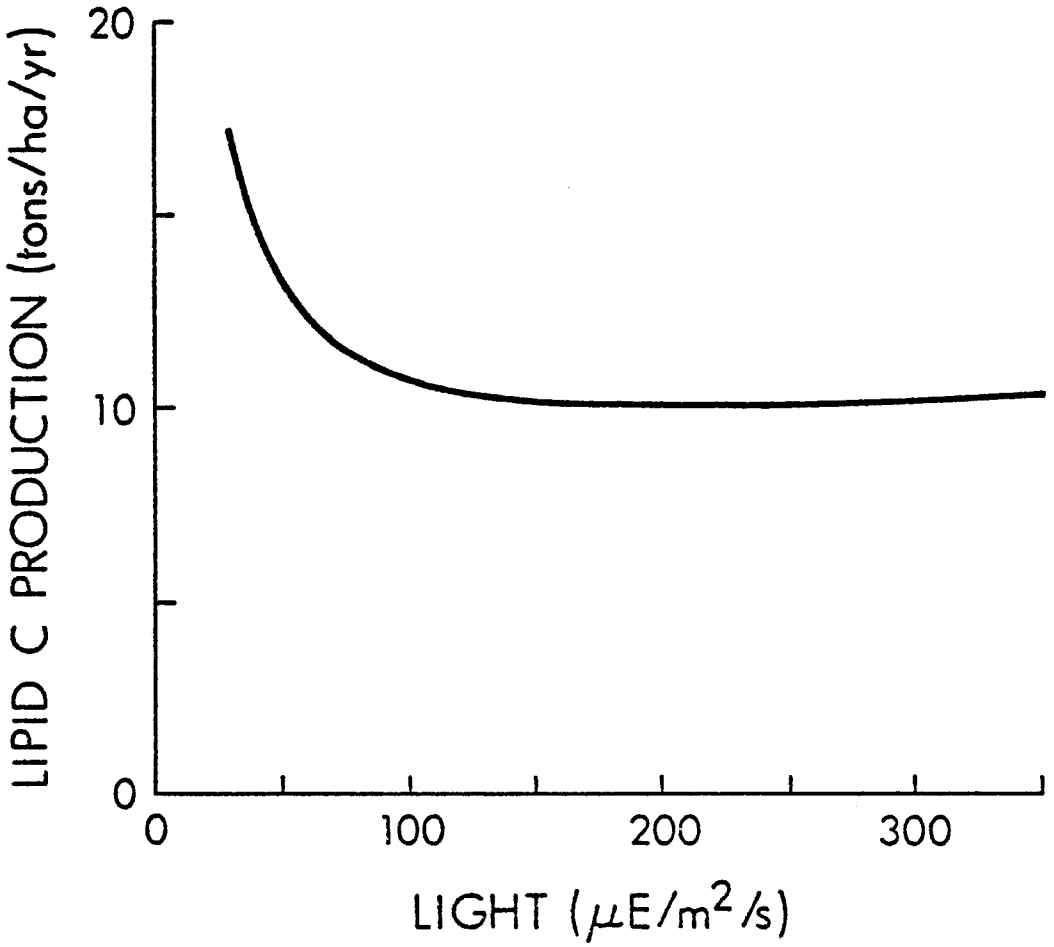


FIGURE 12. LIPID CARBON PRODUCTION AS A FUNCTION OF LIGHT INTENSITY IN A NUTRIENT-LIMITED POPULATION OF THE WOODS HOLE STRAIN GROWING AT 0.41 PER DAY.

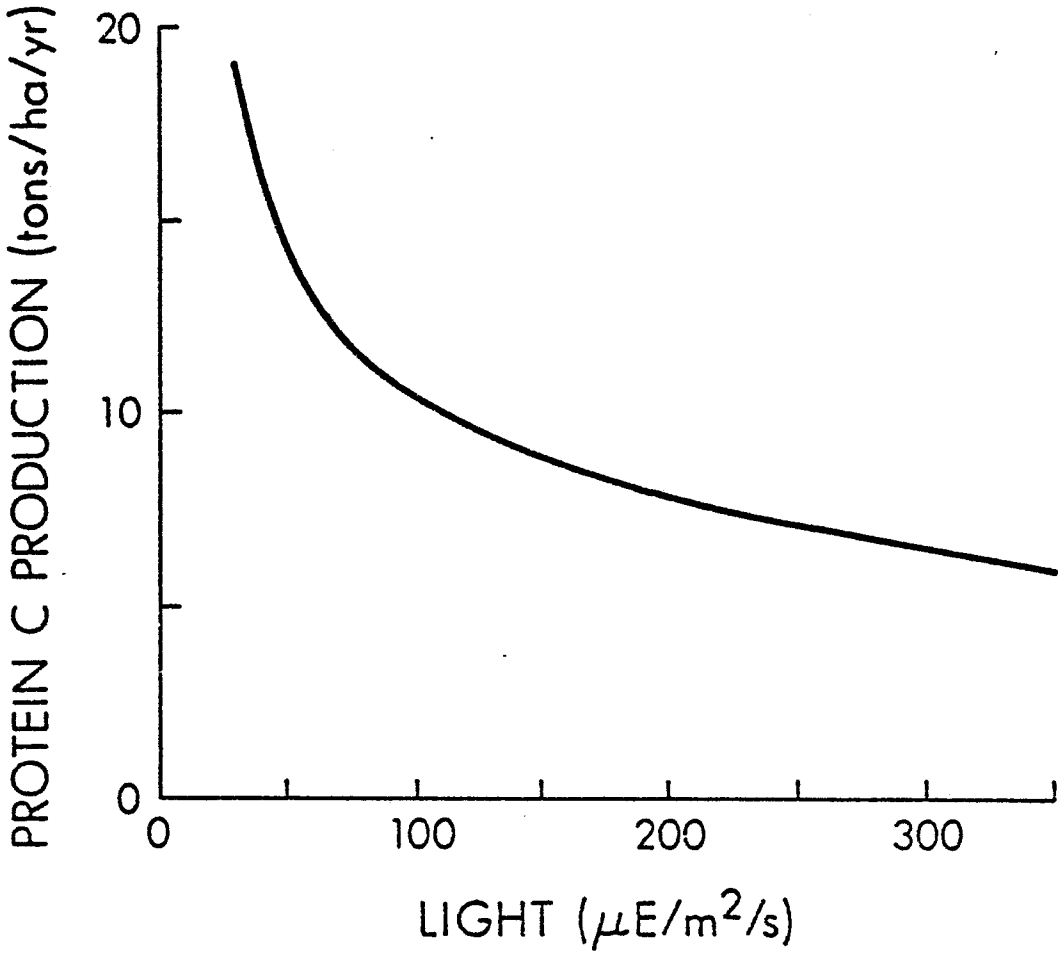


FIGURE 13. PROTEIN CARBON PRODUCTION AS A FUNCTION OF LIGHT INTENSITY IN A NUTRIENT-LIMITED POPULATION OF THE WOODS HOLE STRAIN GROWING AT 0.41 PER DAY.

reported. Maximum growth rate at I = 300 $\mu Ein \cdot m^{-2} \cdot s^{-1}$ is about 1.05 d^{-1} and in theory production curves based on nutrient-limited and light-limited data should merge at that growth rate. Figures 14-16 show the relevant results for the WH strain. In all three cases, maximum production was achieved under light limitation, the optimal growth rate being about 0.4 d^{-1}. The crossing of the total C and protein C production curves at growth rates slightly less than 1.0 d^{-1} reflects errors in the curve fitting process; as noted the curves should merge at a growth rate of 1.05 d^{-1}.

6. Conclusion

The data not only indicate that protein and lipid production are maximized in a light-limited system, but that production of both is maximized at essentially the same light intensity. While it is true that the compositional characteristics of P. tricornutum vary widely as a function of growth conditions, our results indicate that it would not be econimically or energetically reasonable to vary the growth conditions of P. tricornutum so as to change its composition, because deviation from optimal growth conditions reduces both protein and lipid production. Optimal production is achieved using the Thomas strain at a light-limited growth rate of 0.39 d^{-1}, under which conditions about 31% of the cell's carbon is lipid carbon and about 44% is protein carbon. From an examination of the graphs in Figures 4, 7 and 10, it is apparent that a light intensity slightly below the optimal value can greatly reduce production, and given day-to-day variability in solar insolation it would presumably be desirable to operate an APR somewhat to the right of the optimum light intensity point in order to avoid large reductions in production on cloudy days.

EFFECT OF A $CuSO_4$ FILTER ON LIPID AND PROTEIN PRODUCTION

Early work with the APR utilized a roughly 0.9 cm thick 3% $CuSO_4$ solution to absorb infrared light and thus retard overheating of the culture. Some information suggests that lipid production is stimulated when cells are grown in blue light, and it therefore seems possible that use of $CuSO_4$ filters would serve a dual purpose in the raceway system. The experiments reported here were conducted with the WH strain, using a 10% $CuSO_4 \cdot 5H_2O$ solution about 0.25 cm thick contained in a plastic sandwich.

The results of the study, conducted under nutrient saturated conditions, are shown in Fig. 17. C:Chl *a* ratios and the percentage of protein carbon are increased under blue light, and the percentage of lipid C is slightly reduced. Growth rate as a function of light intensity was virtually indentical under blue and white light. Since introduction of the $CuSO_4$ filter obviously removes some light quanta from the visible spectrum, an allowance must be made for this fact in evaluating the effectiveness of the filter system in stimualting carbon production. Since our filter removed about 40% of incoming visible quanta, the conclusion from Fig. 17 is that at I = 35 $\mu Ein \cdot m^{-2} \cdot s^{-1}$ there would be essentially no net enhancement of lipid production, a 20% enhancement of protein production and a 5% enhancement of total carbon production.

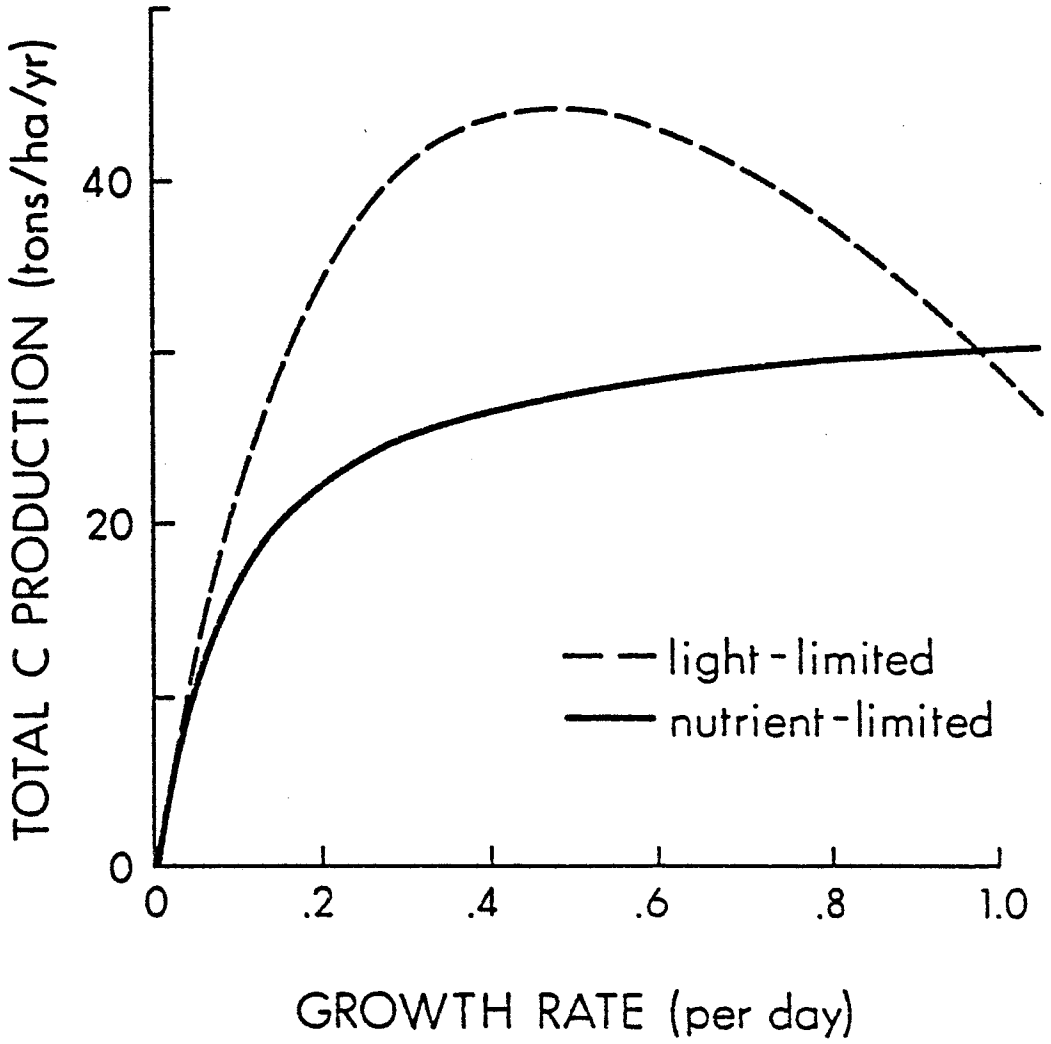


FIGURE 14. TOTAL CARBON PRODUCTION FOR THE WOODS HOLE STRAIN AS A FUNCTION OF GROWTH RATE UNDER LIGHT-LIMITED CONDITIONS, AND UNDER NUTRIENT-LIMITED CONDITIONS AT $I = 300\ \mu E \cdot M^{-2} \cdot S^{-1}$.

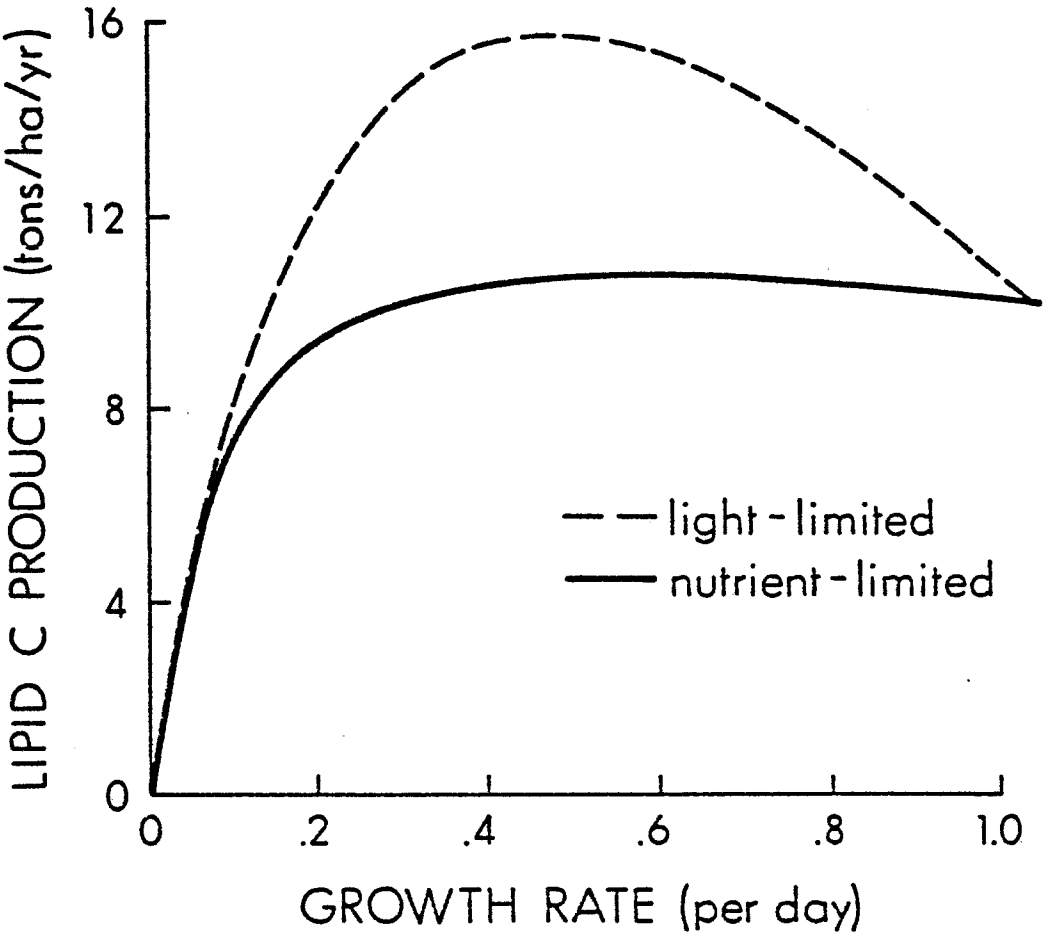


FIGURE 15. LIPID CARBON PRODUCTION FOR THE WOODS HOLE STRAIN AS A FUNCTION OF GROWTH RATE UNDER LIGHT-LIMITED CONDITIONS, AND UNDER NUTRIENT-LIMITED CONDITIONS AT $I = 300\ \mu E \cdot M^{-2} \cdot S^{-1}$.

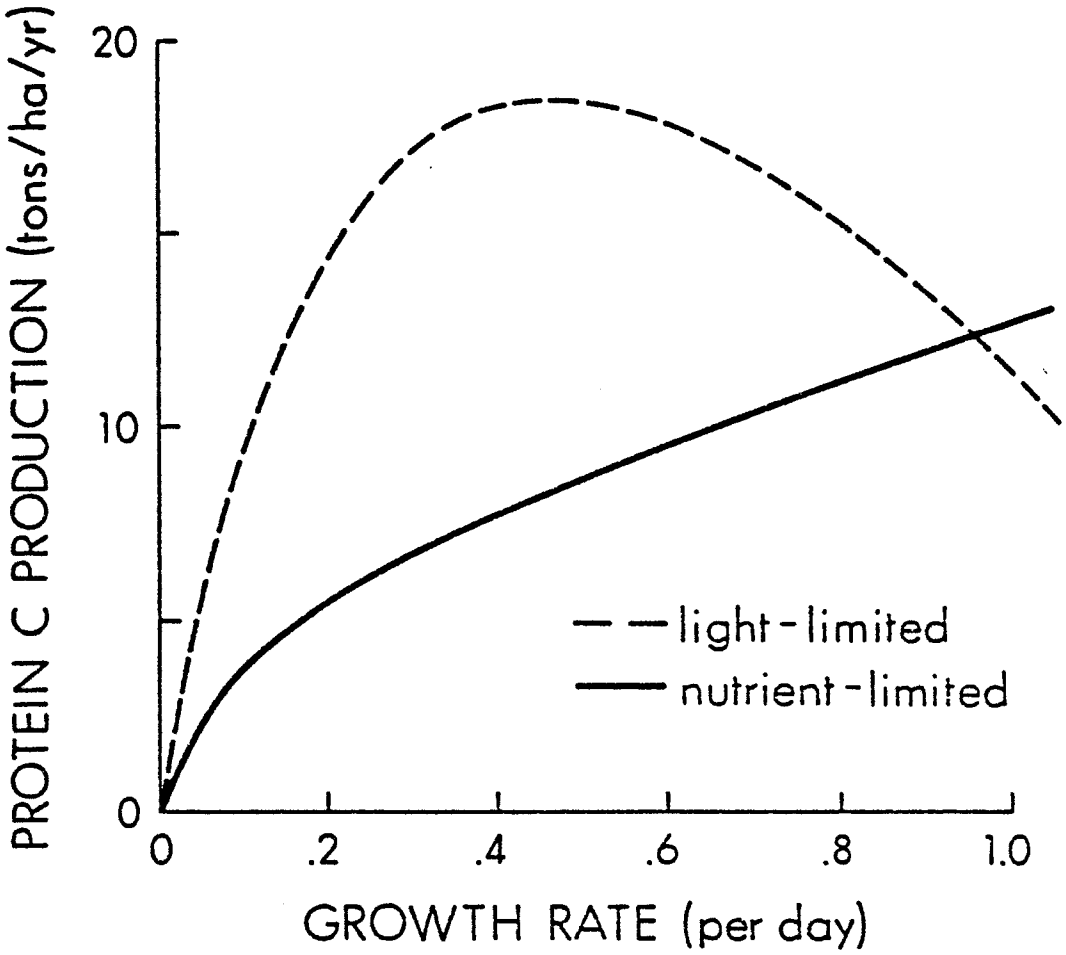


FIGURE 16. PROTEIN CARBON PRODUCTION FOR THE WOODS HOLE STRAIN AS A FUNCTION OF GROWTH RATE UNDER LIGHT-LIMITED CONDITIONS, AND UNDER NUTRIENT-LIMITED CONDITIONS AT $I = 300\ \mu E \cdot M^{-2} \cdot S^{-1}$.

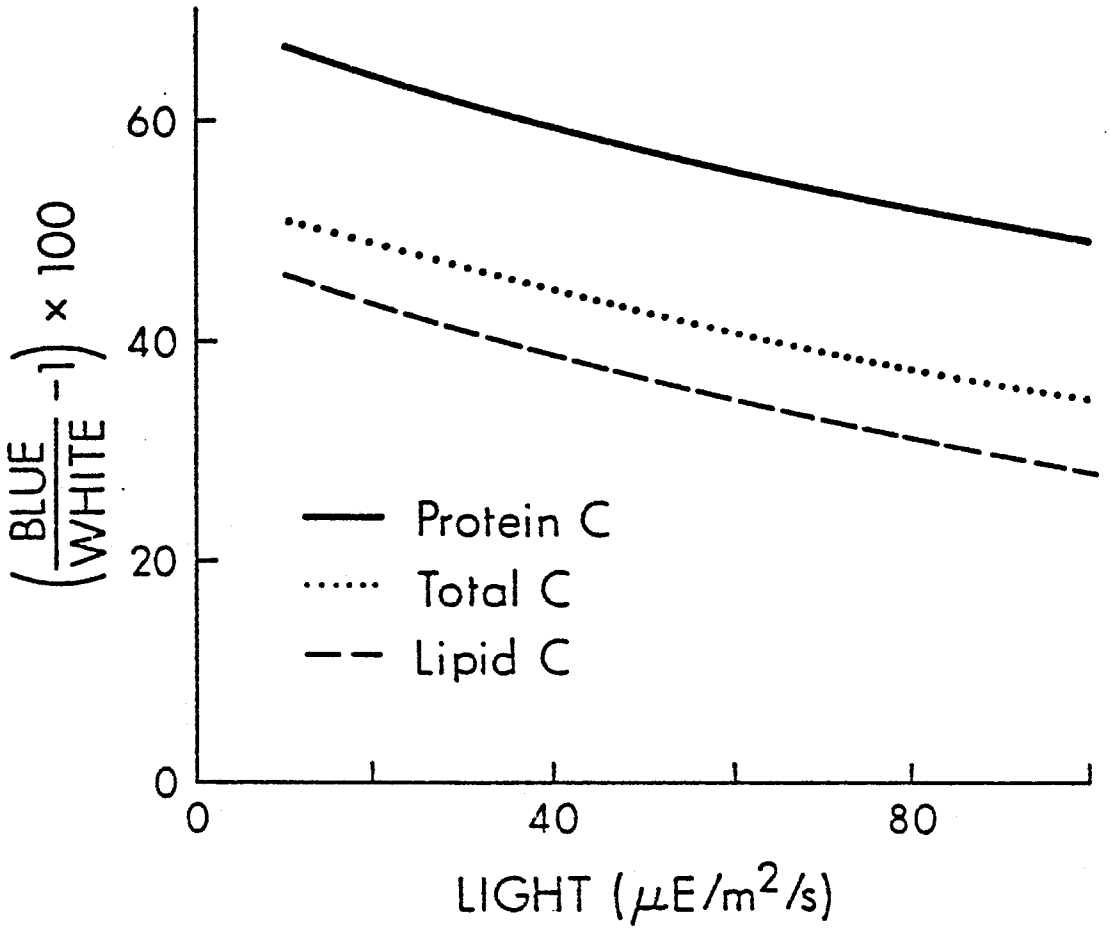


FIGURE 17. RELATIVE PRODUCTION OF CARBON AS A FUNCTION OF LIGHT INTENSITY FOR THE WOODS HOLE STRAIN GROWN UNDER WHITE LIGHT (SPECTRAL COMPOSITION SIMILAR TO SUNLIGHT) AND BLUE LIGHT (WHITE LIGHT WHICH HAD PASSED THROUGH A $CuSO_4$ FILTER).

The conclusion is that while large scale enhancements of carbon production are not likely to result from the use of $CuSO_4$ filters, the filters can probably be designed in a manner which will not reduce production, and will probably lead to a modest increase. Since the filters reduce the management costs of the raceway be retarding overheating of the culture and storing a potentially valuable byproduct (heat), we conclude that installation of $CuSO_4$ filters is advisable in the APR.

EFFECT OF FLASHING LIGHT ON CARBON PRODUCTION

The rationale for conducting these experiments is that efficiency of light utilization is improved if light is provided in alternating light and dark periods rather than continuously. The flashing light effect has been documented for time periods as short as 0.02 sec and as long as several hours. There is also evidence that simply modulating the light intensity about a mean value may produce a 20-80% increase in production versus incubation at the mean light intensity. Although the physiological mechanisms which underlie the flashing light effect probably differ over the wide range of time scales studied, the details of the mechanisms need not concern us. Simulation of a flashing light or modulated light effect in the APR system could greatly enhance production. The idea would be to grow a sufficiently dense and turbulent culture so that vertical mixing would introduce the cells alternately into lighted and darkened regions. Since we anticipated that the time scale of such mixing processes would be on the order of one second, our initial experiments were conducted on that time scale. The cells were exposed to light 50% of the time, and the results compared to those from cells grown on continuous light.

There was little difference in the composition of the cells grown under flashing light and continuous light. The growth rate of the cells grown under flashing light was substantially lower than that of the cells grown under continuous light, but the difference was often less than a factor of two. Based on the composition and growth rate data, production curves under continuous light and the flashing light regime are shown in Fig. 18. In making this comparison, we have multiplied the flashing light production figures by a factor of two, on the assumption that during the dark period the light is used to illuminate a second culture. In effect this calculation amounts to assuming that the culture is separated into two subpopulations, which are alternately illuminated and darkened as the culture is mixed. The results show that production is enhanced at light levels greater than about 125 - 150 $\mu Ein \cdot m^{-2} \cdot s^{-1}$ under the flashing light regime, but that there is a reduction in production at lower levels of flashing light. The increase in production at 200 $\mu Ein \cdot m^{-2} \cdot s^{-1}$ is only 10-15%.

While these experiments do not seem particularly encouraging from the standpoint of increasing production, it is possible that a different light:dark periodicity could significantly change the picture. The present results for example indicate that for $I \cong 1000\ \mu Ein \cdot m^{-2} \cdot s^{-1}$, lipid production under a flashing light regime would be about 70% greater than lipid production under continuous light, and it is therefore conceivable that exposing populations to short pulses of very bright light followed by relatively long periods of darkness could substantially increase production.

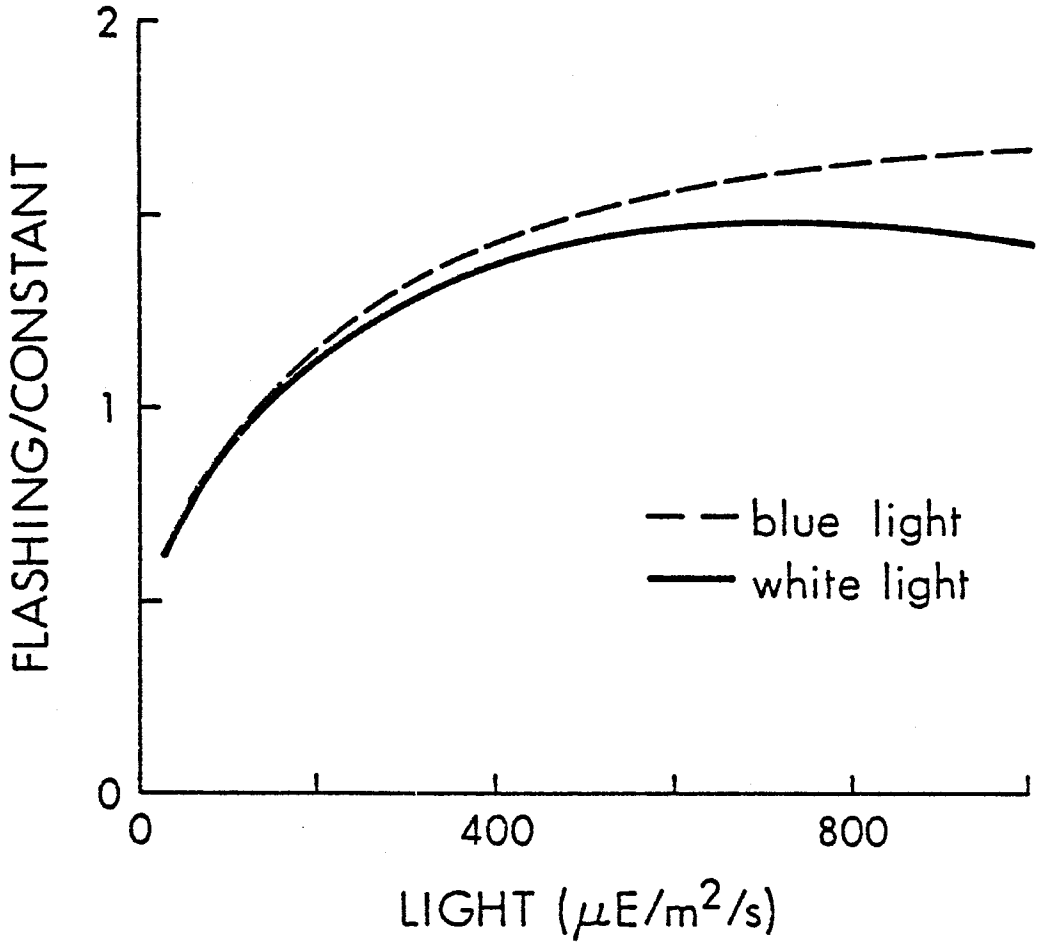


FIGURE 18. RELATIVE PRODUCTION OF CARBON UNDER A FLASHING LIGHT REGIME (50:50 L:D, ONE SECOND PULSE DURATION) VERSUS CONSTANT LIGHT FOR THE WOODS HOLE STRAIN AS A FUNCTION OF LIGHT INTENSITY FOR BOTH BLUE AND WHITE LIGHT.

EFFECTS OF TEMPERATURE ON CARBON PRODUCTION

Growth rate and composition data collected from nutrient-limited populations of P. tricornutum indicate that a temperature of 26.5°C is nearly lethal to P. tricornutum, while a temperature of 24.5°C appears close to optimal in terms of maximizing growth rate. Overheating can obviously be a serious problems in a shallow, dense culture. On the other hand, an effect to cool the raceway to a temperature as low as 20°C for example would be costly. It is therefore desirable to know how protein and lipid production are affected by temperature.

Our results show that the C:Chl a ratio in the cells is negatively correlated with temperature, but that there is very little correlation between either percent lipid or percent protein and temperature in the range $21.5°C \leq T \leq 26.5°C$. As a result, carbon, protein and lipid production are all negatively correlated with temperature, as indicated for lipid production in Fig. 19. These experiments were carried out under nutrient-limited conditions, and it is not clear what effect temperature will have at low light levels ($\sim$35 μEin $\cdot$ $m^{-2} \cdot s^{-1}$) and nutrient-saturating conditions. However, the data in Fig. 19 indicate that at high light levels ($\sim$300 μEin $\cdot$ $m^{-2} \cdot s^{-1}$) and nutrient saturating conditions ($\mu \sim 1.2$ d^{-1}) there is about a 36% increase in lipid production if the temperature is reduced from 24.5°C to 21.5°C.

The optimum temperature at which to operate APR's will evidently represent a trade off between cost of temperature control and the value of increased production. Although temperature control beyond the use of IR filters may be uneconomical in production APR's, temperature considerations may influence the geographical siting of APR's. Furthermore, if a cheap cooling mechanism is available (e.g. OTEC water effluent from a land-based OTEC facility), then more active temperative regulation may be advisable.

APR RESULTS

Table 1 summarizes a few of the results obtained with the APR during a five-day period from 3/18/81 to 3/23/81. During this time period, the APR was diluted at a rate of 0.2 d^{-1}. From the changes in cell numbers, particulate carbon, particulate nitrogen and dry weight, we conclude that the cells were growing at a mean rate of 0.32 - 0.38 d^{-1} during this period. These figures compare reasonably well with the mean growth rate of 0.39 d^{-1} reported from earlier APR prototypes. Using the % protein versus N/C regression in Fig. 20 and the mean N/C ratio of 0.214 from the APR product in Table 2, we conclude that the APR cells' carbon consisted of about 55% protein carbon. Assuming that protein C accounts for 51.3% of the AFDW of protein and that C accounts for 54.93% of the AFDW of P. tricornutum, we conclude that about 58% of the AFDW of these cells consisted of protein. This figure compares well with the value of 63% reported from earlier APR prototypes. An analysis of percent ash on APR samples gave a figure of 37% as compared with a mean value of 35% from the earlier prototype.

Thus in terms of growth rate and cellular composition, the cells in our APR appear very similar to those in earlier APR prototypes. The principal difference in the two cultures has been in the cell densities. Cell densities

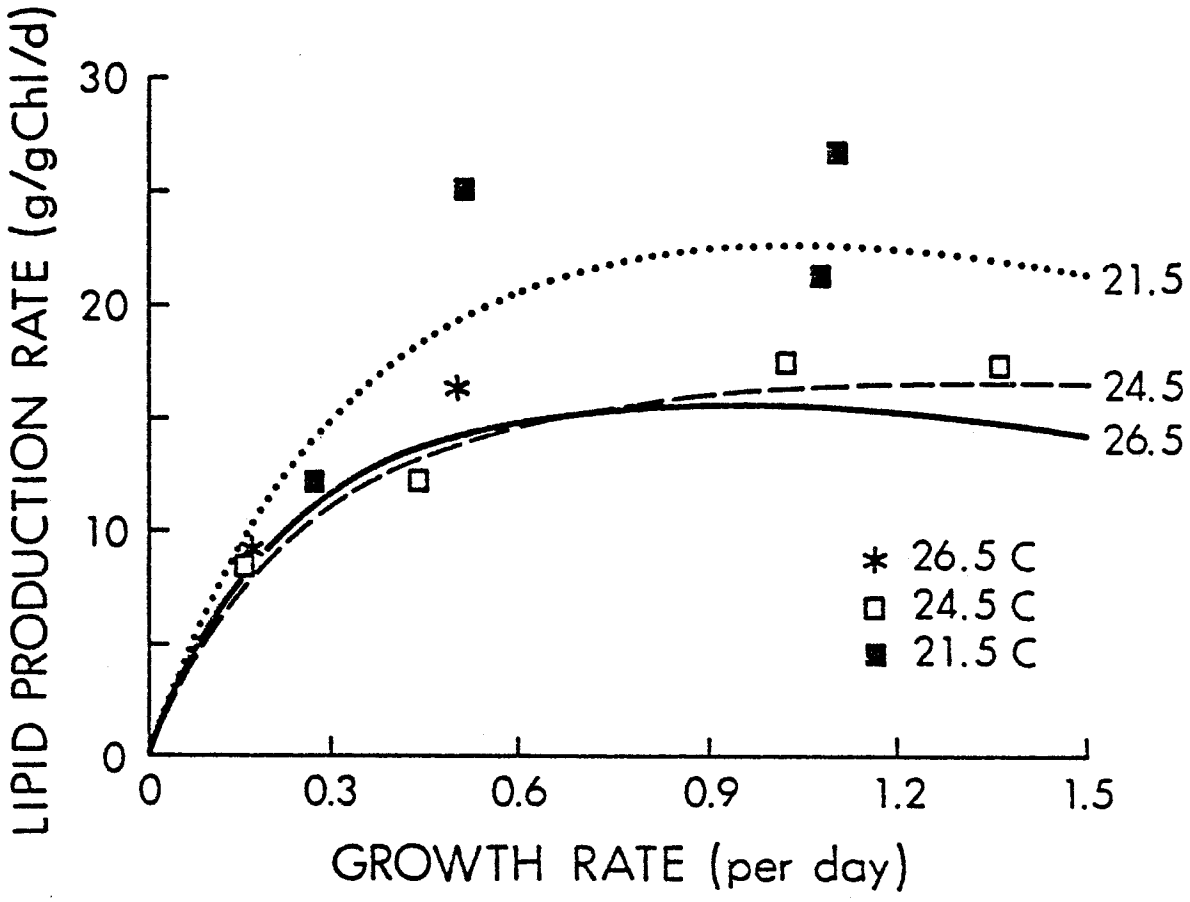


FIGURE 19. EFFECT OF TEMPERATURE ON LIPID PRODUCTION AS A FUNCTION OF NUTRIENT-LIMITED GROWTH RATE FOR THE WOODS HOLE STRAIN.

TABLE 1. PRELIMINARY APR RESULTS

Date	Cells 10^6/mℓ	Part.C µM	Part.N µM	N/C atoms	Chl a µg/ℓ	Dry wt. mg/ℓ	Temp. mean °C	Salinity °/₀₀	pH
3/18	3.06	3926	763	0.194	675	53	20.4	32.9	7.51
3/19	4.46	4978	986	0.198	401	65	22.0	36.9	7.55
3/20	5.02	5235	1278	0.244	460	95	21.5	34.8	7.42
3/21	6.51	5624	1297	0.231	543	82	22.4	35.4	7.52
3/22	6.06	5538	1093	0.197	550	105	22.9	35.8	7.54
3/23	6.04	6590	1447	0.220	572	119	23.4	36.5	7.39

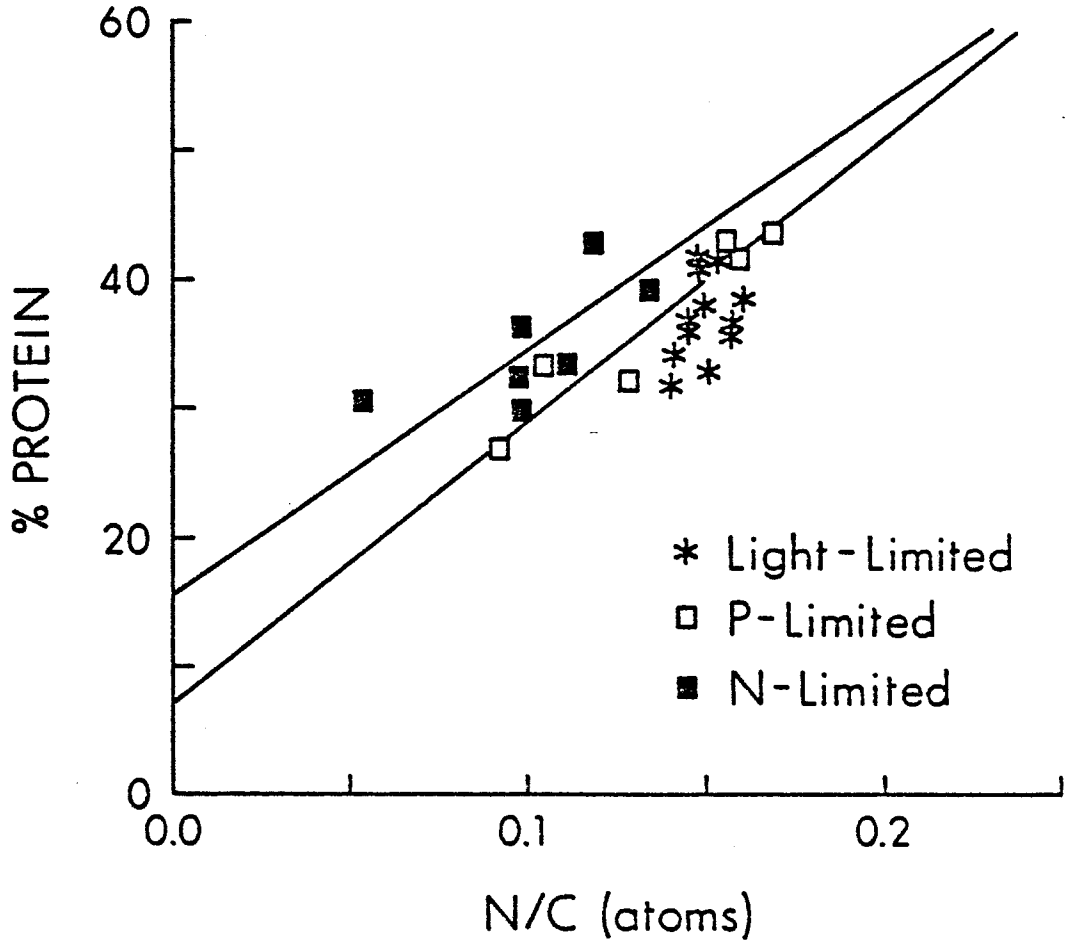


FIGURE 20. CORRELATION BETWEEN PERCENT PROTEIN AND CELLULAR N:C RATIOS FOR THE WOODS HOLE STRAIN. UPPER REGRESSION LINE AND LOWER REGRESSION LINE WERE FIT TO THE N-LIMITED AND P-LIMITED DATA RESPECTIVELY.

in our APR have generally been about $5x10^6$ cells$\cdot ml^{-1}$, while earlier APR's were operated at cell densities of 20-$30x10^6$ cells$\cdot ml^{-1}$.

Let us assume for the moment that we are able to achieve a cell density of about $25x10^6$ cells per ml in the APR. How deep a system would be required to achieve the optimal light intensity of about 40 $\mu E \cdot m^{-2} \cdot s^{-1}$? Based on the data in Table 1, at a cell density of $25x10^6$ cells$\cdot ml^{-1}$ we can expect a Chl a concentration of about 2250 $mg \cdot m^{-3}$. Given this Chl a concentration and assuming a specific extinction coefficient of $22m^2 \cdot g^{-1}$ Chl a. the extinction coefficient of light in the racway should be about $50m^{-1}$ at a cell density of $25x10^6$ cells$\cdot ml^{-1}$. If the incident light intensity is 1200 $\mu E \cdot m^{-2} \cdot s^{-1}$, reduction of the water column mean light intensity to about 40 $\mu E \cdot m^{-2} \cdot s^{-1}$ would require a water column 1200/(40$\cdot$50) = 0.6 m deep. Assuming a surface area for our APR of $50m^2$, we have a total volume in the APR of 3000 liters.

If we assume the population is growing at about 0.4 d^{-1}, the optimum growth rate based on previous calculations, then we need to harvest the cells from (0.40)(3000) = 1200 liters of water per day. We have found that foam is constantly produced in the APR as a result of the aeration system which drives the circulation. The density of cells in this foam is about 40 times the density of cells in the APR. Therefore we need to harvest 1200/(40$\cdot$24) = 1.25 liters$\cdot hr^{-1}$ of foam. We have found that a simple commercial liquid vacuum system will remove over 2ℓ per hour of foam if operated about 10% of the time. Thus the amount of foam generated at a single point in the raceway appears more than adequate to provide the harvesting mechanism we need. The material collected from this foam can be easily dried in the sun in a matter of hours to yield a product suitable for harvesting.

We therefore feel that we have identified a suitable harvesting mechanism. There is no doubt that generation of foam can be effected at numerous points in the APR by means of air bubbling, and the efficiency of foam production can be increased with the use of air stones to produce fine bubbles. At present the foam is trapped with a weir device and removed by periodic suction, but it is likely that in a production APR a skimmer would prove to be more energy efficient in harvesting the foam than our suction device. We have continued to use the suction system for the present, as it is more easily adapted to changes in APR design.

ECONOMIC ANALYSIS

Our chemostat results indicate that under optimum production conditions we can expect to produce 0.92 bbl of free oils and 0.47 short tons of protein for every short ton of AFDW produced by the APR. Costs of operating mass culture systems vary greatly, from about $800 per acre-year to $18,000 per acre-year. The original APR proposal assumed an operating cost of $12,401 per acre-year. Table 2 shows the projected break-even prices of the oil product assuming operating costs of $12,401 and $18,000 per acre-year as a function of total biomass production (AFDW). In making this analysis, we have assumed a value for the protein byproduct of $248 per short ton as in the original APR proposal.

The results indicate that at an operating cost of $12,401 per acre-year, oil

TABLE 2. BREAK-EVEN COST OF OIL PRODUCED IN APR SYSTEM. COLUMNS A AND B ASSUME OPERATING COSTS OF $12,401 and $18,000 PER ACRE-YEAR RESPECTIVELY.

Total Production (tons AFDW/acre-year)	Protein Production (tons AFDW/acre-year)	Oil Production (bbl/acre-year)	Break Even Cost($/bbl) A	B
20	9.4	18.4	547	852
30	14.1	27.6	323	526
40	18.8	36.8	210	362
50	23.5	46.0	143	265
60	28.2	55.2	98	199
70	32.9	64.4	66	153
80	37.6	73.6	42	118
90	42.3	82.8	23	91
100	47.0	92.0	8	69

can be produced at a cost comparable to today's prices if total production is approximately 80 short tons AFDW per acre-year. If production costs reach $18,000 per acre-year, then competitive prices would not be achieved even at a total production rate of 100 short tons AFDW per acre-year. At this point one may be guardedly optimistic in assuming that a total production rate of 80 short tons AFDW per acre-year can be achieved. This figure translates into 179 metric tons AFDW per ha-yr, 2-3 times the levels of production typically achieved in mass culture systems. However, the figures is only about 35% higher than the optimum production based on chemostat work. It is possible that the effects of modulated blue light on the system will allow the extra production to be achieved.

EFFECTS OF LIGHT INTENSITY AND NITROGEN DEFICIENCY ON YIELD, PROXIMATE COMPOSITION, AND PHOTOSYNTHETIC EFFICIENCY OF *PHAEODACTYLUM*

W. H. Thomas, D. L. R. Seibert, M. Alden, and A. Neori
Institute of Marine Resources
Scripps Institution of Oceanography
University of California, San Diego
La Jolla, CA 92093

ABSTRACT

Phaeodactylum tricornutum Bohlin was grown in a large 5-cm thick culture container in seawater enriched with N, P and trace metals. The culture was dense (~1000 mg dry weight liter^{-1}) and was grown at three light intensities (39.5, 59.6, and 69.8% of maximum sunlight at La Jolla) in the batch culture mode. The lowest intensity gave the highest yield (21.74 gm m^{-2}day^{-1}) and efficiency of photosynthetically active (400-700 nm) light utilization (12.23%). Other intensities were slightly or greatly photoinhibitory. Continuous culture under N-sufficient conditions resulted in a somewhat lower yield and efficiency than in batch culture. The proximate composition of N-sufficient cells was about 60% protein, 10% carbohydrate, 20% lipid and 10-17% ash. Nitrogen deficiency greatly reduced yield, efficiency, and protein content. Lipid content was increased to 30% of the dry weight. We conclude that, because of the reduced overall cellular yield, increased lipid content does not result in an increased lipid yield in *Phaeodactylum*. These results are compared with other investigations both in the laboratory and in outdoor mass cultures.

INTRODUCTION

One of the general goals of the Solar Energy Research Institute's Aquatic Biomass Program is to promote the use of microalgae as a source of energy and organic products. Outdoor mass cultures are envisioned in which algae will utilize sunlight at a high efficiency as compared with land plants (see Wassinck *et al.*, [1]). Much research on the production of valuable cellular products by mass culture has been performed with freshwater microalgae, principally *Chlorella* [2, 3, 4, 5], but little work has been done on the capabilities of marine microalgae. This is somewhat surprising since seawater is an abundant source of water and certain major nutrients (K, Mg, Ca, S, etc.) and coastal areas, particularly in the tropics, often receive a great deal of sunlight on a yearly basis. Furthermore, marine microalgae may be adaptable to growth in other sources of saline water such as those often found in desert areas.

The purpose of our project is to study marine microalgae (phytoplankton) in the laboratory to provide essential basic information useful for establishing successful outdoor mass cultures that will produce a maximum amount of cellular energy and valuable byproducts. We are mimicing, insofar as possible, natural sunlight, and are screening a number of marine species for

their ability to incorporate light energy at high efficiencies into a cellular product that is high in total energy (calories) and in lipid. We are testing the effects of nitrogen deficiency and, to a certain extent, light intensity, in manipulating the cells toward these goals. In addition to analysis of harvested cells for total lipid and caloric content, we are analyzing for other proximate constituents such as protein, carbohydrate, carbon, nitrogen, and ash.

This paper reports results with the first marine microalgal species that we have investigated, *Phaeodactylum tricornutum* Bohlin. We chose this species because it is easy to grow relative to others; a great deal is already known about its biology, chemical composition, physiology, and biochemistry; and it is being used as a test organism by another SERI subcontractor in Hawaii.

MATERIAL AND METHODS

The Microalga

We have cultured *Phaeodactylum tricornutum* in our laboratory for many years. This alga was provided to us by Dr. David Leighton and probably came from the University of California Marine Laboratory at Bodega Bay. However, its precise origin and strain designation are unknown.

Medium

Phaeodactylum was grown in filtered (0.45-μm pore size) Scripps Pier seawater enriched with 15 mg-at N $liter^{-1}$ as $(NH_4)_2SO_4$, 1.25 mg-at P $liter^{-1}$ as K_2HPO_4, and the trace metal mixture in medium "f" [6]. For N-deficient medium the $(NH_4)_2SO_4$ enrichment was deleted.

Preculture

Prior to the main experiment, the alga was precultured to a high density in a nine-liter serum bottle incubated at 21°C and a continuous, incident light intensity of 0.056 cal $cm^{-2}min^{-1}$ in the photosynthetically active spectral range (400-700 nm). The light was supplied by a bank of fluorescent lamps. The culture was bubbled with a 1% CO_2-in-air at a rate of 2000 mℓ min^{-1}. After a cell density of 6.72 X 10^6 cells $mℓ^{-1}$ was reached, 3.4 liters of this preculture was poured aseptically into the main culture apparatus.

Light Supply

The main culture apparatus is diagrammed in Figure 1. The light source was a 2000-watt tungsten-halide lamp (3200°K) mounted in a 50-cm diameter parabolic reflector such as is generally used for stage lighting. The lamp was operated diurnally (12:12 L/D cycle). When the lamp was turned on, voltage was applied gradually over a one-minute period and was controlled by an automatic dimmer connected to a timing circuit. This prolonged lamp life beyond the rated 500 hours to >1100 hours so that one bulb was used throughout the

experiment (94 days). The light was filtered through a 7-cm thickness of flowing tapwater and a 3-cm thickness of 3% $CuSO_4$ to remove infra-red radiation. Figure 2 shows spectral distributions (350-800 nm) of light energy (3 intensities) at the culture surface with and without the $CuSO_4$ filter. The latter filter removed red and far-red irradiance quite effectively. These spectral distributions were measured with an Optronics 741-V spectroradiometer. Light intensity was not uniform over the whole surface of the culture; at the medium light level it varied from 0.095 to 0.247 cal $cm^{-2}min^{-1}$ in the photosynthetically active spectral range (400-700 nm) as measured at 143 points over the culture area with a Li-Cor 190 SE flat response energy sensor. These 143 intensities were integrated over the whole culture surface area (705.3 cm^2) to obtain values for total calories received by the culture per 12-hour light period. These values were 62,715, 94,661, and 109,405 calories per culture per light period for the three light intensities used. In 1967 Strickland [7] measured total daily sunlight radiation at La Jolla from April to September. The maximum value was 450 cal $cm^{-2}day^{-1}$. Over the area of our culture (705.3 cm^2) this would be 317,389 cal day^{-1} of which one-half (158,695 cal day^{-1}) would be in the 400-700 nm spectral range. Dividing the cal day^{-1} figures for low, medium, and high intensities for the culture by 158,695 cal day^{-1} yields values for the percentage of maximum sunlight at La Jolla supplied to our culture. These were 39.5, 59.6, and 68.9%, respectively, for the three intensities. The medium light intensity was used for most of the experiment. The lower intensity was achieved by placing a plastic diffusing screen over the $CuSO_4$ filter; and the higher intensity was achieved by moving the light source to 8 cm from the tapwater filter rather than the 20 cm distance shown in Figure 1. The spectral distribution of the light was not changed by varying these intensities (see Figure 2). At a given location at the culture surface light intensities integrated from 400-700 nm by the spectroradiometer were within 5% of those measured with the Li-Cor sensor. Daily measurements at the location of maximum intensity (I_0) with the Li-Cor sensor established that the lamp output did not vary appreciably (<10%) over the 94-day experiment. Measurements (Li-Cor sensor) were also made of light (I) transmitted through the 5 cm culture thickness to roughly follow culture growth. During the experiment the logarithm of I/I_0 correlated very well with cellular dry weight per liter ($r = 0.856$).

The Culture

Figure 1 shows the side configuration of the main culture container which was constructed of clear plexiglass. It was 5 cm thick, 39 cm deep, and 24 cm wide. The bottom was curved for ease of mixing. The container was filled to a depth of 28 cm with 3.4 liters of preculture, after sterilization of the container with 70% ethanol followed by a rinse with sterile deionized water. An aeration (1% CO_2-in-air at 2000 $m\ell$ min^{-1}) tube reached the bottom of the culture and aeration mixed the culture vigorously. The gas was washed by bubbling through sterile deionized water. An overflow and pressure-release tube extended to the culture surface; overflow regulated the culture volume at 3.4 liters. A sampling tube extended into the center of the culture. Sampling and harvesting were carried out by clamping off

FIGURE 1. DIAGRAM OF CONTINUOUS CULTURE APPARATUS.

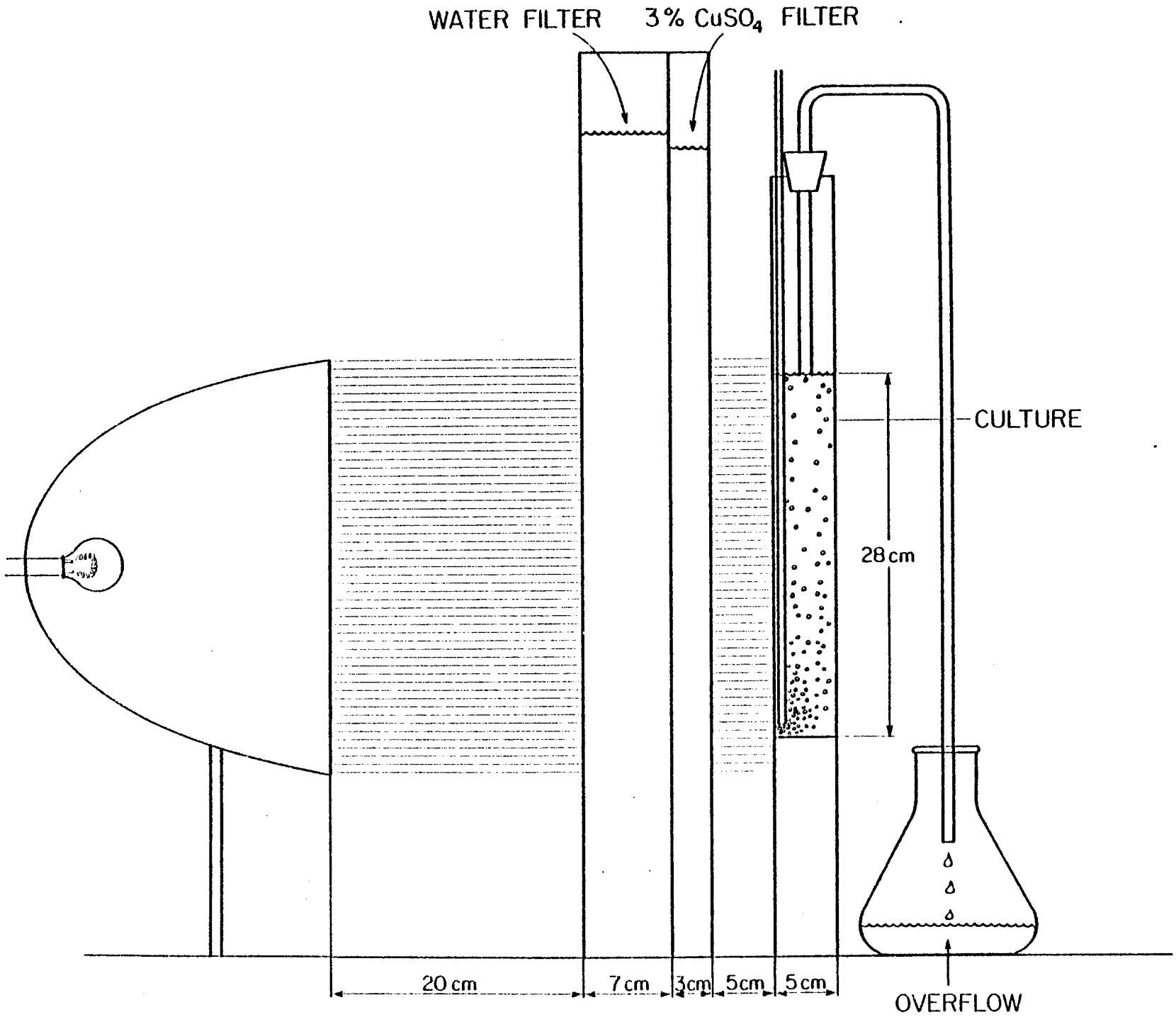
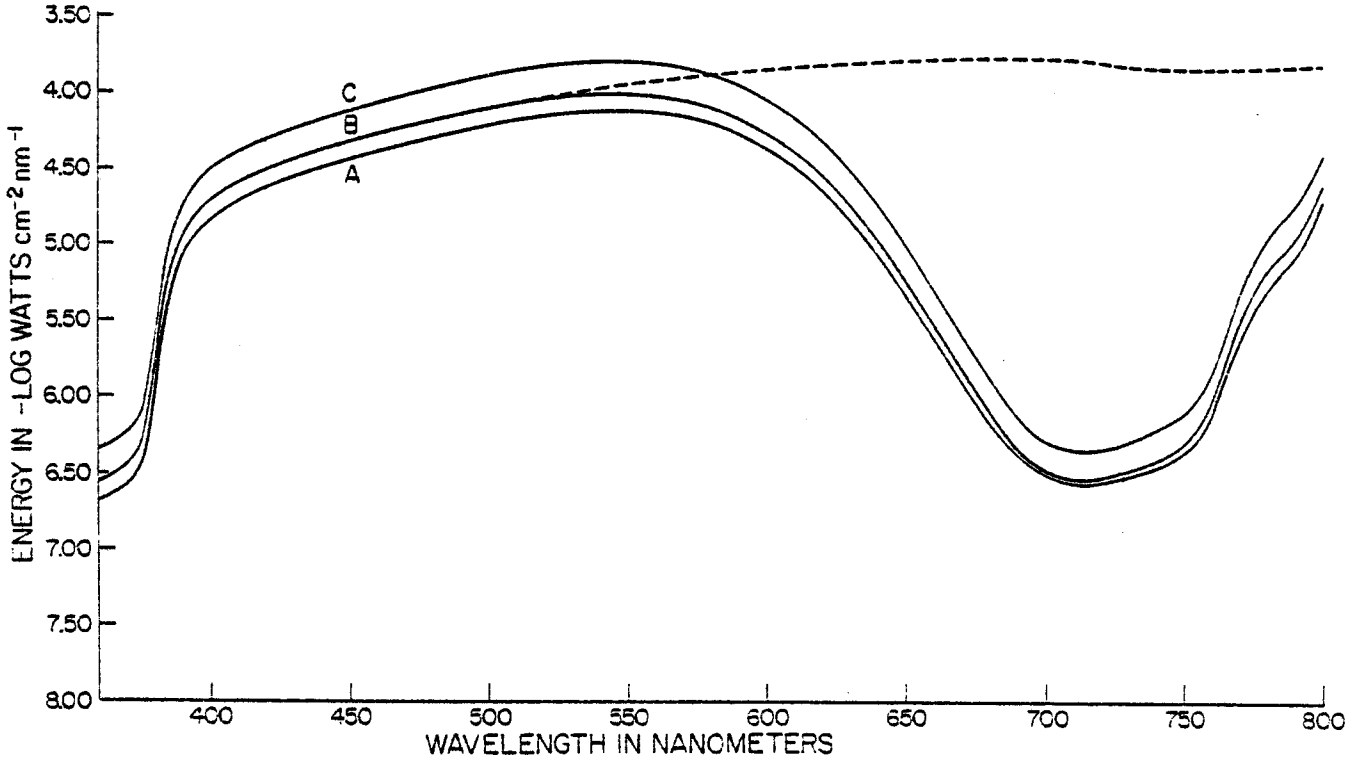

WATER FILTER
3% $CuSO_4$ FILTER
CULTURE
28 cm
20 cm
7 cm
3cm
5cm
5cm
OVERFLOW

FIGURE 2. SPECTRAL DISTRIBUTION OF FILTERED 3200°K LIGHT SUPPLIED TO MAIN CULTURE. A: LOW INTENSITY, 62,715 CAL DAY^{-1}; B: MEDIUM INTENSITY, 94,661 CAL DAY^{-1}; C: HIGH INTENSITY, 109,405 CAL DAY^{-1}. THE DASHED LINE SHOWS THE DISTRIBUTION OF B WITH THE $CuSO_4$ FILTER REMOVED AND WITH FILTRATION ONLY THROUGH FLOWING TAPWATER.


3.50
4.00
4.50
5.00
5.50
6.00
6.50
7.00
7.50
8.00
ENERGY IN -LOG WATTS cm^{-2} nm^{-1}
C
B
A
400
450
500
550
600
650
700
750
800
WAVELENGTH IN NANOMETERS

the overflow tube so that air pressure forced the sample out into a collection container. A fourth tube did not reach into the culture and was for the addition of medium via peristaltic pumping from a 20-liter glass carboy. The medium consisted of sterile-filtered Scripps Pier seawater enriched as described above. It was continuously mixed by aeration. The culture and medium were in contact only with plexiglass plastic, Pyrex glass, and silicone rubber. While temperature-controlled water was circulated through a cooling jacket surrounding the sides of the culture container, this was not adequate to control culture temperature and temperature was principally controlled by placing the entire apparatus, including the lamp, in a cold room set at 20°C. During the 94-day experiment the culture temperature was 22.7 ± 1.0°C as measured daily two hours after the light came on. Because temperature control could only be achieved with the cold room air-conditioner, we did not attempt to vary temperature diurnally.

Sampling, Harvesting, and Growth Measurements

Daily sampling for routine growth measurements was carried out over the full 94 days of the experiment by withdrawing <100 mℓ of cell suspension. periodically larger amounts (300-700 mℓ) were withdrawn as harvests for proximate and caloric analyses. Portions of each harvest (240 mℓ or greater) were centrifuged at 1270 g for 20 minutes. The centrifuged cells were washed once with deionized water and dried at 60°C. Other portions of the harvest or sample were filtered on glass fiber filters for proximate analyses and dry weight determinations. The dry weight samples were dried at 60°C and the other samples were frozen for later analysis.

Growth was measured by cell numbers, optical density, and dry weight. Cells were counted in diluted (filtered seawater) suspensions with a Model B Coulter Counter. Optical density was measured at 750 nm in a 1 cm spectrophotometer cell. For dry weights, filters were weighed on a Cahn microbalance. The weights were corrected for adsorbed salts by weighing blank filters through which filtered seawater had been run. Cell numbers correlated well ($r = 0.821$) with dry weights throughout the experiment as did optical densities ($r = 0.867$). pH of the cell suspension was measured daily or oftener. Due to NH_4^+ uptake, pH was sometimes reduced to values <5.0. It was adjusted back to levels >7.0 by addition of 1M KOH.

Analytical Methods

Total cellular carbon, nitrogen and hydrogen analyses were performed with a Hewlett-Packard Model 185 CHN analyzer [8]. Cellular protein was calculated from the %N value X 6.25. Cellular carbohydrate was analyzed by the phenol-H_2SO_4 spectrophotometric method of Myklestad and Haug [9]. To ensure that polysaccharide was hydrolyzed completely by the 80% H_2SO_4, the samples were heated for one hour at 100°C and then allowed to stand at room temperature for 24 hours. Lipid was extracted from frozen filtered cells by 2:1 (V/V) methanol-chloroform at 40°C [10]. The extract was washed with deionized water to remove water-soluble material and an aliquot was dried at 40°C in a teflon cup and weighed on the microbalance. Considerable methanol-chloroform

extractable material was removed by the water wash and the lipid values would have been much higher if the wash had not been done. After their initial weighing, dry weight filters were heated at 450°C overnight and reweighed to determine ash content. Ash was also determined by weighing the residue from the combustion of caloric samples and by combustion of 5-10 mg pellets of cells at 450°C. Caloric values were determined with a Phillipson microbomb calorimeter in dried, centrifuged cells. The calorimeter was connected to a recorder and to a millivoltmeter and the apparatus was standardized by combusting known weights of benzoic acid. Cells were filtered and frozen for pigment analyses, but these have not yet been completed. During the development of N-deficiency (attained by pumping in N-free medium), NH_4^+ in the supernatant from centrifuged samples was measured with a Lazar NH_4^+ electrode.

Analytical Precision

Five separate samples were filtered for each analysis. Precision (standard deviation/mean) was 6.3%, 5.8%, 18.9%, 18.6%, 11.9%, <1%, and 2.3% for analyses of carbon, nitrogen (and protein), hydrogen, carbohydrate, lipid, cell numbers, and dry weight, respectively. Only a few replicates were determined for calories and ash. The "±" values reported are standard deviations.

RESULTS

Light Intensity Effects on Batch Culture (N-Sufficiency)

Initially the culture was grown in batch mode without dilution at a light intensity chosen to approximate 50% sunlight. The actual intensity was about 60% of the maximum summer intensity at La Jolla [7]. At the end of the experiment the effects of two additional intensities were tested.

Figure 3 shows increases in dry weight at the three intensities. Increases in cell numbers and optical density were also linear. Table 1 gives the various culture parameters at these intensities. The maximum growth rate and yield occurred at the lowest intensity; these decreased slightly at the medium intensity; and apparent photoinhibition occurred at the highest intensity. The efficiency of light utilization was maximal at the lowest intensity and decreased greatly with increasing intensity. Although yield decreased only slightly at the medium intensity, the efficiency was much less than that at low intensity since more light was probably being supplied than could be used and there may have been slight photoinhibition at this medium intensity.

At the three light intensities there were no large differences in the proximate chemical composition of the crop nor in caloric content. The yield of lipid at low, medium, and high intensities was calculated to be 5.63, 4.11, and 3.21 gm $m^{-2}day^{-1}$, respectively; the yield of protein was 12.98, 12.08, and 10.05 gm $m^{-2}day^{-1}$, respectively, at the three intensities.

FIGURE 3. GROWTH OF *PHAEODACTYLUM* AT THREE LIGHT INTENSITIES. A: AT 62,715 CAL. DAY^{-1}; B: AT 94,661 CAL. DAY^{-1}; C: AT 109,405 CAL. DAY^{-1}. THE GROWTH CURVES ARE DISPLACED FOR CLARITY.

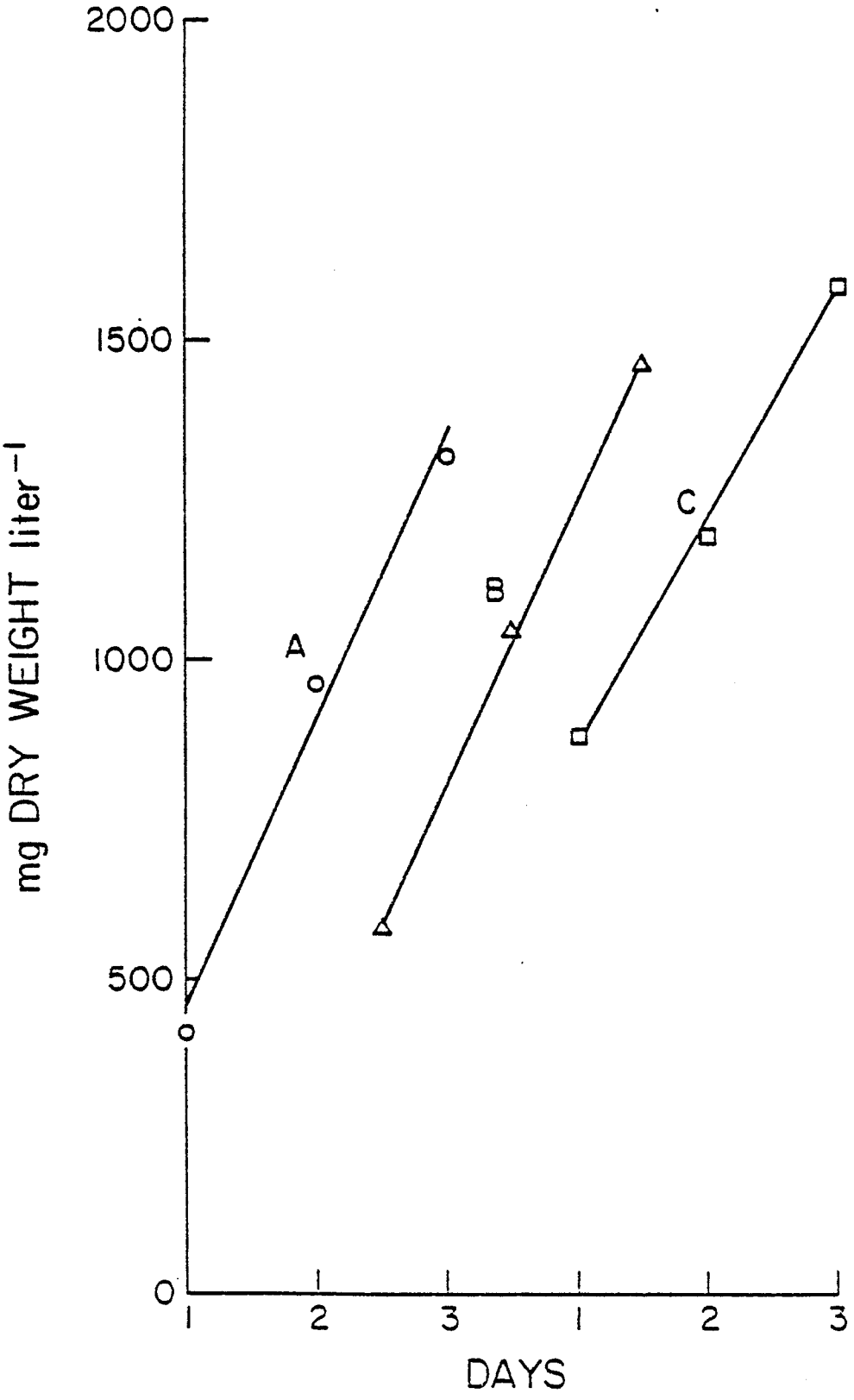

2000
1500
1000
500
0
mg DRY WEIGHT liter⁻¹
A
B
C
1
2
3
1
2
3
DAYS

TABLE 1. *PHAEODACTYLUM* BATCH CULTURE PARAMETERS AT THREE LIGHT INTENSITIES AND NITROGEN SUFFICIENCY.

	Batch Culture		
	A	B	C
Culture Dates	2/28-3/2/81	12/12-12/14/80	3/3-3/5/81
Light**:			
Intensity	Low	Medium	High
Cal culture^{-1}day^{-1}	62,715	94,661	109,405
% of maximum La Jolla sunlight	39.5	59.6	68.9
Growth:			
Rate (mg liter^{-1}day^{-1})	451	439	354
Yield (gm m^{-2}day^{-1})	21.74	21.16	17.09
Proximate Composition:			
Carbon (% dry weight)	48.4 ± 5.9	46.8 ± 3.5*	42.6 ± 1.7
Nitrogen (% dry weight)	9.6 ± 0.8	9.1 ± 0.6*	9.4 ± 0.8
Hydrogen (% dry weight)	14.0 ± 3.4	15.3 ± 6.9*	23.9 ± 19.9
Protein (% dry weight)	59.7 ± 5.3	57.1 ± 3.6*	58.8 ± 5.3
Carbohydrate (% dry weight)	13.0 ± 3.1	10.5 ± 1.4*	11.3 ± 1.7
Lipid (% dry weight)	25.9 ± 3.1	19.4 ± 2.1*	18.8 ± 0.9
Ash (% dry weight)	16.9	10.6*	15.5
Energy Content:			
Calories mg dry weight^{-1}	4.97	5.09*	4.93
Calories mg C^{-1}	10.27	10.55*	11.57
Efficiency**:	12.23	8.09	3.49

* Proximate composition and calories from continuous culture (N sufficiency) on 12/29/80 and 1/5/81 samples.

** Light values are for photosynthetically active radiation (400-700 nm) and efficiencies are calculated only for that part of the spectrum.

Continuous Culture (Medium Light Intensity, N-Sufficiency, 0.48 Volume Day^{-1})

Following the batch culture at medium light intensity, N-enriched medium was pumped into the culture at a nominal rate of 0.5 culture volume day^{-1} and the culture overflow was collected. This part of the experiment lasted 19 days. The mean overflow volume was 1628 ± 99 mℓ day^{-1}; this gave an actual dilution rate of 0.48 volume day^{-1}. The mean dry weight of cells was 702 ± 47 mg liter^{-1}. The mean optical density was 2.23 ± 0.42 and the mean cell number was 12.47 ± 1.61 X 10^6 cells mℓ$^{-1}$. From the overflow volume and dry weight values a yield of 16.20 gm m^{-2}day^{-1} was calculated. The proximate composition of the cells was 48.2 ± 2.6%C, 9.4 ± 0.2%N, 17.9 ± 5.5%H, 15.5% ash, 58.9 ±1.1% protein, 11.2 ± 0.8% carbohydrate, and 19.9 ± 3.5% lipid. The yield of protein, carbohydrate, and lipid would thus be 9.54, 1.81, and 3.22 gm m^{-2} day^{-1}, respectively. The caloric value of the cell material was 5.09 cal mg^{-1}dry weight^{-1}. The culture produced 1143 mg dry weight day^{-1} or 5813 cellular calories day^{-1} and adsorbed 99.0% of the light that was supplied or 93714 calories day^{-1}. Thus the photosynthetic efficiency was 6.20%.

At medium light intensity with N-sufficiency, batch culture gave a higher yield and efficiency than continuous culture -- 21.21 gm m^{-2}day^{-1} and 8.09%. We might have been able to dilute the culture slightly faster and achieve still higher yields and efficiencies in the continuous mode of operation, but determining the proper balance between dilution with N-enriched medium and growth to maintain a maximum yield is difficult when growth proceeds in a linear manner. The dilution rate for matching continuous culture to batch culture is highly dependent on the dry weight liter^{-1}. At the crop weight we found (702 mg liter^{-1}) the culture could have been diluted at 0.625 volumes day^{-1} to attain a yield equivalent to that in batch culture. Later on in the experiment, diluting at 0.75 volume day^{-1} resulted in washout of the cells so this latter rate was too high.

Development of N-Deficiency (Medium Light Intensity, Continuous Culture, 0.48 Volume Day^{-1})

Nitrogen deficiency was induced by diluting the culture at the same rate as before with medium to which no NH_4^+ was added. At Day 1 the NH_4^+ concentration in the culture medium was 11.0 mg-at N liter^{-1} (see Figure 4). Since the complete medium contained 15 mg-at N liter^{-1}, the difference should have been 4 mg-at N liter^{-1} in the cells. Calculation of the cellular N from the dry weight and %N value for this day gave a value of 4.1 mg-at cellular N liter^{-1}. Thus there was good agreement in total N in the cells as determined in two independent ways. The NH_4^+ concentration in solution decreased exponentially to zero (<0.0001 mg-at NH_4^+-N liter^{-1}) at Day 4 of deficiency (Figure 4).

Figure 4 also shows changes in crop parameters. Dry weight and cell numbers remained nearly constant until the NH_4^+ was used up and thereafter decreased. Optical density decreased throughout the period of dilution with -N medium.

FIGURE 4. CHANGES IN *PHAEODACTYLUM* CROP PARAMETERS AND IN NH_4^+ CONCENTRATION DURING THE DEVELOPMENT OF NITROGEN DEFICIENCY (MEDIUM LIGHT INTENSITY, CONTINUOUS CULTURE AT 0.48 VOLUMES DAY^{-1}).

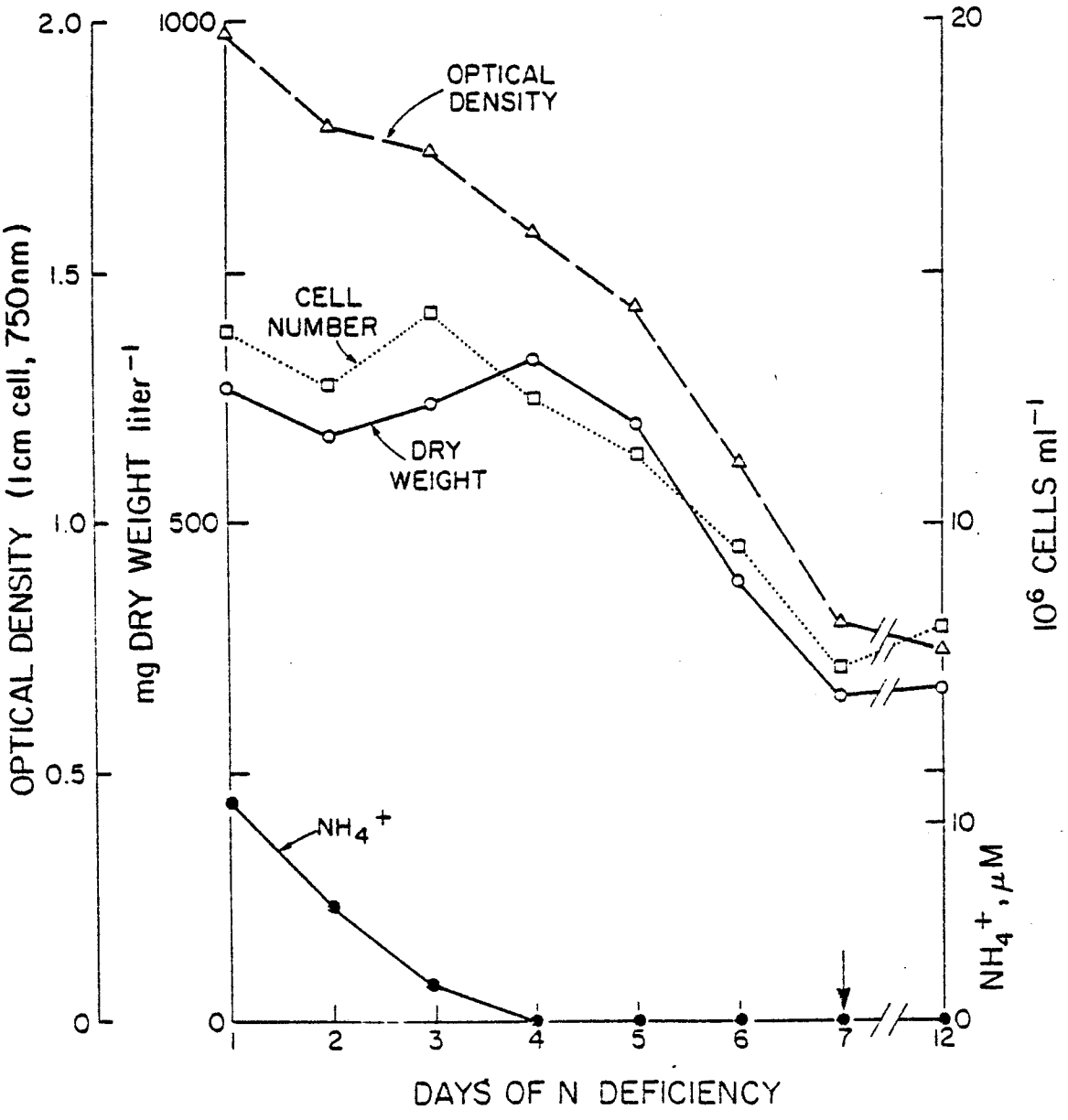

OPTICAL DENSITY (1cm cell, 750nm)
mg DRY WEIGHT liter⁻¹
10⁶ CELLS ml⁻¹
NH4+, μM
OPTICAL DENSITY
CELL NUMBER
DRY WEIGHT
NH4+
DAYS OF N DEFICIENCY
2.0
1.5
1.0
0.5
0
1000
500
0
20
10
10
0
1
2
3
4
5
6
7
12

Table 2 shows the yields as deficiency developed. These were determined by multiplying the dry weight liter^{-1} by the overflow volume in liters. Yields decreased greatly after the NH_4^+ level in solution dropped to zero and cellular N decreased. Also shown in Table 2 are cellular caloric values and efficiencies of light utilization. Caloric content increased somewhat when N-deficiency was pronounced due to increased lipid content (see Figure 6), although the caloric increase was not as dramatic as the decrease in yield and N content. Calories per unit carbon did not change with N-deficiency. The mean value was 9.93 ± 1.52 calories mg C^{-1} during the whole development of deficiency. Efficiencies of light utilization were calculated from the yield, caloric content, and light absorbed by the culture. Efficiencies did not change greatly with decreasing cellular N since caloric content increased as yield decreased. The mean yield of lipid in N-sufficient cells (Days 1-4) was 2.95 gm m^{-2}day^{-1}; that in N-deficient cells (Days 5-7) was 2.69 gm m^{-2} day^{-1}. Thus even though the lipid content increased with N-deficiency (Figure 6), the yield of lipid was about the same as in N-sufficient cells. However mean protein yield decreased greatly; it was 7.59 gm m^{-2}day^{-1} in N-sufficient cells and 2.34 gm m^{-2}day^{-1} in deficient cells.

Figures 5 and 6 show the changes in cellular proximate composition with the development of N-deficiency. Cellular N and protein decreased greatly after NH_4^+ was used up (Day 4). Cellular C did not change greatly nor did ash content. Cellular carbohydrate increased significantly ($p < 0.01$) on Day 6 and then decreased. Cellular lipid increased significantly ($p < 0.01$) on Days 6 and 7. With N-deficiency, photosynthetically fixed carbon may be shunted first into carbohydrate and then into lipid, in contrast to the formation of the main metabolic product, protein, in N-sufficient cells. The increased lipid content and corresponding degree of reduction in the cells was paralleled by an increase in cellular H on Day 7. Extreme deficiency (Day 12) did not result in maintenance of a high lipid or H content even though cellular N was further decreased, and caloric content was also increased. Caloric samples from that day were "oily to the touch" and we cannot explain why the lipid and H contents did not remain high.

In healthy cells, the cytoplasm was dispersed throughout the cell while in N-deficient cells the cytoplasm was clumped in the center of the cell. Oil droplets were possibly present in N-deficient cells, but we are not entirely sure of this point. Cell size did not change with N-deficiency nor did dry weight cell^{-1}. The latter was 56.6 ± 8.4 picograms cell^{-1} in N-sufficient cells and under -N conditions it was 50.5 ± 9.2 picograms cell^{-1}. In all of our microscopic observations cells were single and fusiform; we never saw triradiate cells.

Recovery From N-Deficiency

To allow the cells to recover from deficiency we pumped N-enriched medium into the culture. Recovery was slow and growth did not keep up with dilution for several days. Therefore we stopped pumping and allowed the cells to recover under batch culture conditions. It still took 10 days for the cells to fully recover, at which time cellular composition was nearly identical to that measured earlier in healthy cells.

TABLE 2. YIELDS, CALORIC CONTENT, AND EFFICIENCIES OF LIGHT UTILIZATION AS PHAEODACTYLUM CONTINUOUS CULTURES WERE SHIFTED TO NITROGEN DEFICIENCY (MEDIUM LIGHT INTENSITY, 0.48 VOLUMES DAY^{-1}).

Date	Day	Cellular N Content (% of dry weight)	Calories mg $dry\ weight^{-1}$	Calories mg C^{-1}	Efficiency (%)	Yield (gm $dry\ weight\ m^{-2}\ day^{-1}$)	Lipid Yield (gm $dry\ weight\ m^{-2}\ day^{-1}$)	Protein Yield (gm $dry\ weight\ m^{-2}\ day^{-1}$)
1/5/81	1	9.1	4.92	10.51	5.43	14.61	2.91	8.34
1/6/81	2	9.6	3.37	6.17	3.50	13.70	2.73	8.51
1/7/81	3	8.7	4.39	9.75	4.81	14.48	2.82	7.83
1/8/81	4	5.7	4.24	9.98	5.01	15.85	3.72	5.64
1/9/81	5	3.2	3.69	11.46	4.05	13.96	2.79	2.83
1/10/81	6	3.9	5.53	10.93	4.91	10.43	2.89	2.51
1/11/81	7	3.5	5.48	10.15	4.09	7.75	2.39	1.67
1/16/81	12	2.5	5.73	10.48	*	*	*	*

* Extreme N-deficiency; no dilution (pumping); no growth; yields and efficiency could not be calculated.

FIGURE 5. CHANGES IN % C, N, AND H DURING THE DEVELOPMENT OF NITROGEN DEFICIENCY IN *PHAEODACTYLUM* (MEDIUM LIGHT INTENSITY, CONTINUOUS CULTURE AT 0.48 VOLUMES DAY^{-1} UNTIL DAY 7 -- SEE ARROW).

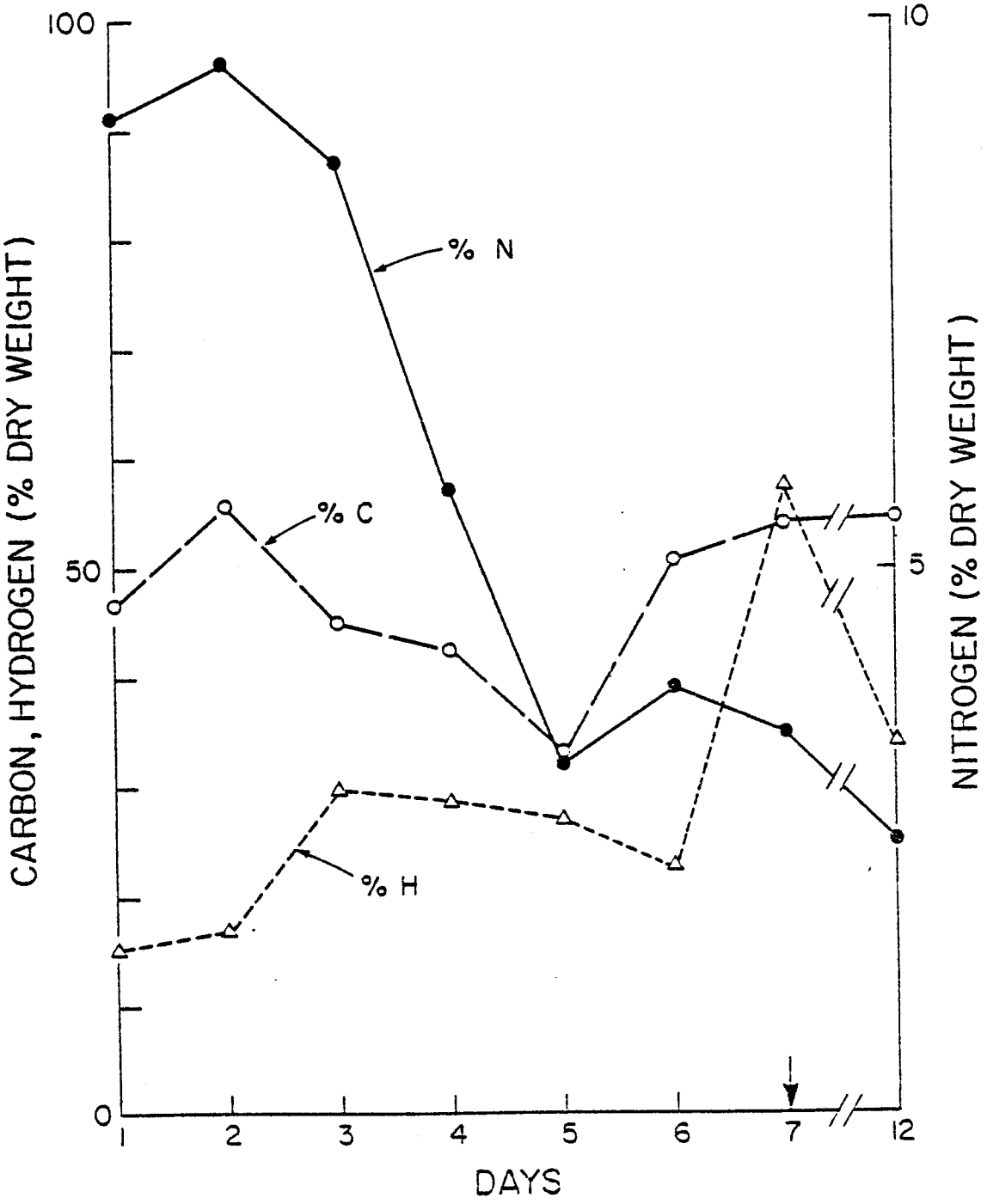
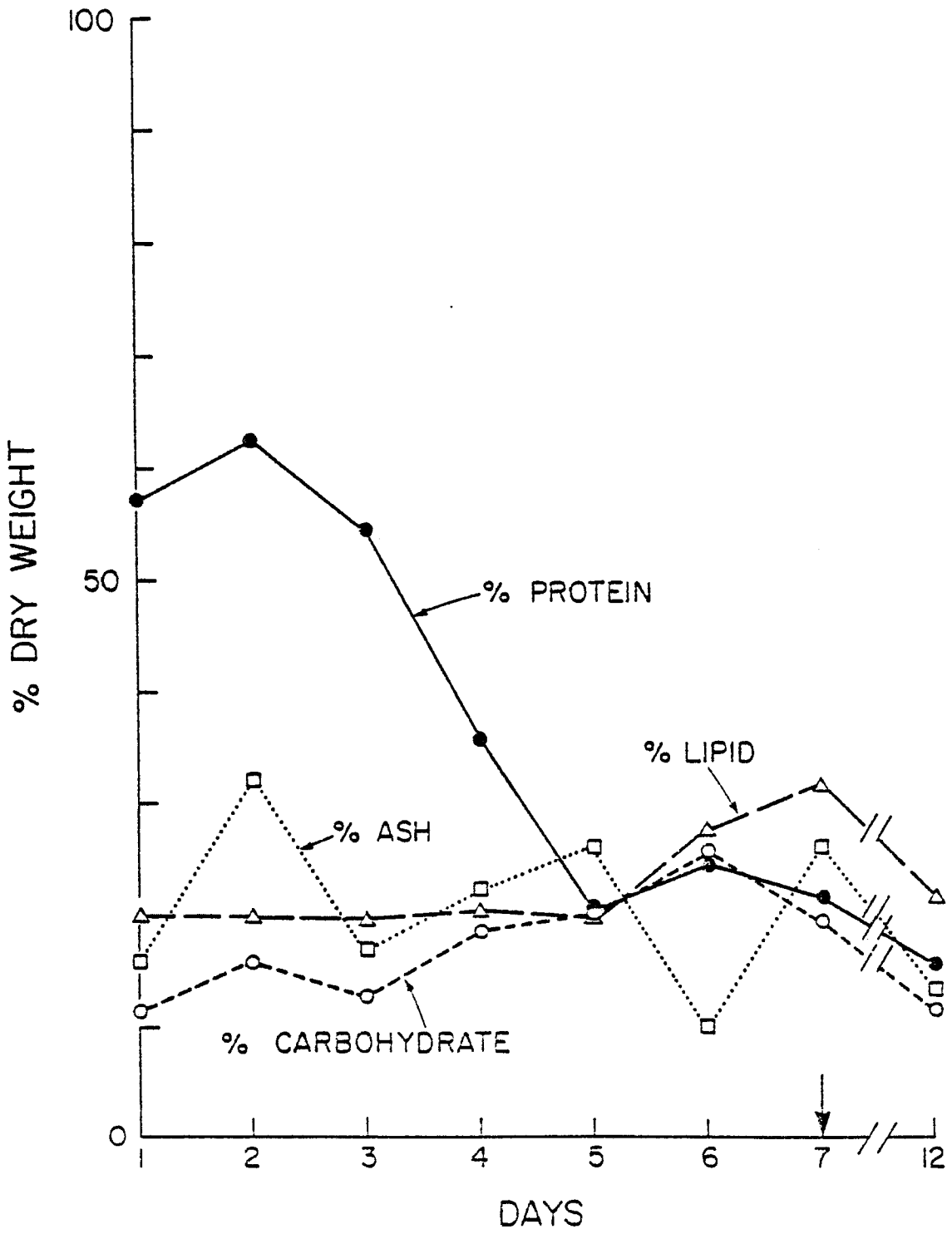

100
50
0
10
5
% N
% C
% H
CARBON, HYDROGEN (% DRY WEIGHT)
NITROGEN (% DRY WEIGHT)
1
2
3
4
5
6
7
12
DAYS

FIGURE 6. CHANGES IN % PROTEIN, CARBOHYDRATE, LIPID, AND ASH DURING THE DEVELOPMENT OF NITROGEN DEFICIENCY IN *PHAEODACTYLUM* (MEDIUM LIGHT INTENSITY, CONTINUOUS CULTURE AT 0.48 VOLUMES DAY^{-1} UNTIL DAY 7 -- SEE ARROW).


100
50
0
% DRY WEIGHT
% PROTEIN
% LIPID
% ASH
% CARBOHYDRATE
1
2
3
4
5
6
7
12
DAYS

Continuous Culture at 0.25 Volumes Day^{-1} Under +N and -N Conditions (Medium Light Intensity)

Following recovery we pumped +N medium into the culture at an actual dilution rate of 0.25 volumes day^{-1} to see if dry weight liter^{-1} would increase enough to increase yield over that at 0.48 volumes day^{-1}. Dry weight did increase to 1045 ± 16 mg liter^{-1} over that observed at 0.48 volumes day^{-1} (702 ± 47 mg liter^{-1}) but the yield at the new dilution rate was only 11.59 gm m^{-2} day^{-1} as compared with 16.20 gm m^{-2}day^{-1} at 0.48 volumes day^{-1}.

We then took the culture through an N-deficiency cycle by diluting with -N medium at this new rate. Full deficiency took an additional day to achieve (to % N = 3.1) as compared with that during the former dilution rate and the effects of deficiency were not as marked as before. That is, lipid increased from 19.7% of the dry weight to only 23.2% and caloric values increased from 4.91 calories mg^{-1} to 5.31 calories mg^{-1}. Protein, of course, decreased greatly -- from 58.3% to 19.7%, but other constituents did not change appreciably. Yield went from 11.59 gm m^{-2}day^{-1} (+N) to 7.11 gm m^{-2}day^{-1} (full deficiency). Efficiency of light utilization went from 4.16% (+N) to 3.19% (-N).

It seems apparent that diluting at this rate was very much sub-optimal as compared with 0.48 volumes day^{-1} (+N conditions) where the yield was 16.20 gm m^{-2}day^{-1} and the efficiency was 6.20% (see above).

DISCUSSION

When we planned this experiment, we opted for a light intensity close to 50% of that of sunlight, since this was what might be received by outdoor mass cultures screened by some sort of an infra-red absorbing filter, i.e. a $CuSO_4$ solution. The actual intensity during most of the experiment was about 60% of the maximum summer sunlight intensity at La Jolla. This was probably slightly inhibitory and definite photoinhibition was found at an intensity of 69% of sunlight (see Figure 3 and Table 1).

Mann and Myers [11] determined that photosynthesis (O_2 evolution; 1 cm culture thickness, 40 mg dry weight liter^{-1}) in *Phaeodactylum* was saturated at an intensity of 0.057 cal cm^{-2}min^{-1} or for our culture vessel area and over a whole 12-hour day, 28,945 cal culture^{-1}day^{-1}. Goldman [12] gives a general curve (his Figure 11, top curve) for yield versus light intensity at a similar saturation intensity (I_s = 0.06 cal cm^{-2}min^{-1}), but in that curve does not consider photoinhibition. At our low intensity (62,715 cal culture^{-1}day^{-1}) the yield from his curve would be approximately 22 gm m^{-2}day^{-1}. In batch culture we obtained a yield of 21.74 gm m^{-2}day^{-1} at this intensity. It is probable, that in our well-mixed, 5-cm thick culture with a dry weight approximately 25 times that used by Mann and Myers, the mean light seen by the cells was very close to the saturation intensity they found for a thinner, less dense culture. The agreement in these comparisons is remarkable since they measured short-term photosynthesis and we measured increases in dry weight

over a two-day period. Using Goldman's curve to calculate yield at our medium and high intensities, the values would be approximately 30 and 35 gm $m^{-2}day^{-1}$, respectively. We attribute our lower yields (Table 1) to photoinhibition. Further empirical studies are needed on the effect of intensity on yield and efficiency in dense cell suspensions with a culture vessel such as ours (or in a thicker culture vessel); such studies would have obvious applications to outdoor mass culturing.

Goldman [13] has summarized maximum and average yields of various algae in many outdoor mass culture studies up to 1977. The maximum values range from 11-35 gm $m^{-2}day^{-1}$ and the average values range from 2-27 gm $m^{-2}day^{-1}$. In a later paper [14] he states that maximum yields of 30-40 gm $m^{-2}day^{-1}$ can be expected under ideal conditions. A maximum yield of 35.6 gm $m^{-2}day^{-1}$ for sewage-grown algae in the summer of 1979 in Israel was found by Shelef *et al.* [15]. Their yearly average was 25.2 gm $m^{-2}day^{-1}$. The maximum yield was found at a pond depth of 25 cm and without a light filter to remove infra-red irradiance. Yields were reduced by greater pond depth. Further yield values are given by various authors in reference 5. With *Phaeodactylum*, Ansell *et al.* [16] reported a yield of 8 gm $m^{-2}day^{-1}$ in outdoor culture and Raymond [17] obtained a yield of 41 gm $m^{-2}day^{-1}$ in an outdoor 5-cm thick culture in Hawaii. Our yield at the lowest light intensity in batch culture under nutrient-sufficient conditions was 21.74 gm $m^{-2}day^{-1}$. This value is within the range of those reported above. We wonder, if the culture thickness were varied and the truly optimal light intensity was determined for a given thickness, that *Phaeodactylum* could not be "pushed" further. To do this we might also increase the nutrient concentration in the medium. The nitrogen content of our present medium would support a crop of 2200 mg $liter^{-1}$. It seems necessary to obtain a balance between the medium, culture thickness, light intensity, and culture dry weight to push *Phaeodactylum* to its maximum yield. The same considerations would be necessary to push any alga and such data would be useful in outdoor culture applications.

The efficiency of light utilization by *Phaeodactylum* in our culture system varied greatly with culture conditions (light intensity, N supply, etc.). However our maximum efficiency -- 12.23% utilization of photosynthetically active radiation -- is very close to a value of 13% reported by Raymond [17] for this species in outdoor culture. Goldman [12] suggests that the maximum theoretical efficiency would be about 20%. Here again, variation of culture thickness, cell density, light intensity supplied, and nutrient supply might increase the actual measured efficiency.

In fusiform *Phaeodactylum* cells Lewin *et al.* [18] reported that the protein, carbohydrate, lipid, and ash contents were 41%, 2%, 34%, and 12% of the dry weight, respectively, and that miscellaneous water- or acid-soluble compounds contributed an additional 12%. Their culture conditions are not stated. In healthy, exponentially-growing *Phaeodactylum* cells, Parsons *et al.* [19] reported that protein, carbohydrate, fat, and ash made up 33%, 24%, 6.6%, and 7.6% of the dry weight, respectively. Our values for protein (~60%) in N-sufficient cells were much higher than those previously reported. Our carbohydrate (~10%) and lipid (~20%) values were intermediate between the previous

values; and our ash values (10-17%) are similar to those reported by Lewin *et al.* and somewhat higher than those given by Parsons *et al.*

In our N-sufficient cells the sum of the percentages of protein, carbohydrate, lipid, and ash was generally near 100%. Lewin *et al.* [18] also reported a recovery near 100% but added an unknown water- and acid-soluble fraction to the protein, carbohydrate, lipid and ash percentages to calculate this recovery. Parsons *et al.* [19] obtained a recovery of 73% but did not include such a soluble fraction. In our N-deficient cells recoveries were generally low (60-85%). We did not routinely measure the water-soluble polar material obtained after methanol-chloroform extraction of lipid samples, but a few weighings of this material indicated that it was an appreciable fraction. It may increase in N-deficient cells; would increase total recovery; and would be measured along with the non-polar lipids if we had not washed the methanol-chloroform extract.

We obtained values for calories per carbon of around 10-11 calories mg C^{-1}. These are similar to the general value of 11.4 calories mg C^{-1} found by Platt and Irwin [20]. Our values for this relationship did not change with nitrogen deficiency.

Nitrogen deficiency resulted in a large decrease in protein content and an increase in lipid content. However, N limitation greatly decreased the overall dry weight yield so that lipid yield was not increased even though cellular lipid content went up. Thus, for this species, we conclude that manipulation of cellular N content is not the best procedure to maximize lipid yield.

ACKNOWLEDGMENTS

We are grateful to Mr. Sandor Kaupp for the spectral distribution measurements. This work was supported by Contract No. XK-09111-1 from the Solar Energy Research Institute.

REFERENCES

1. Wassinck, E.C., Kok, B., and van Oorschot, J.L.P.: The efficiency of light-energy conversion in *Chlorella* cultures as compared with higher plants. In Burlew, J.S. (ed.) Algal Culture. From Laboratory to Pilot Plant, Carnegie Inst. Washington Publ. No. 600, Washington, D.C., 1953, pp. 56-62.

2. Burlew, J.S. (ed.): Algal Culture. From Laboratory to Pilot Plant, Carnegie Inst. Washington Publ. No. 600, Washington, D.C., 1953, 356 pp.

3. Tamiya, H.: Mass culture of algae. Ann. Rev. Plant Physiol., 8, 309-333 (1957).

4. Soeder, C.J. and Binsack, R. (eds.): Microalgae for Food and Feed. A Status Analysis. Arch. Hydrobiol. Beih. Ergebn. Limnol., Heft 11, 300 pp. (1978).

5. Shelef, G. and Soeder, C.J. (eds.): Algae Biomass. Production and Use, Elsevier/North Holland Biomedical Press, Amsterdam, 1980, 852 pp.

6. Guillard, R.R.L.: Culture of phytoplankton for feeding marine invertebrates. In Smith, W.L. and Chanley, M.H. (eds.), Culture of Marine Invertebrate Animals, Plenum Publishing Co., N.Y., 1975, pp. 29-60.

7. Strickland, J.D.H. (ed.): The Ecology of the Plankton off La Jolla, California, in the Period April Through September, 1967. Bull. Scripps Inst. Oceanogr., Vol. 17, University of California Press, Berkeley, 1970, 103 pp.

8. Sharp, J.H.: Improved analysis for "particulate" organic carbon and nitrogen from seawater. Limnol. Oceanogr., 19, 984-989 (1974).

9. Myklestad, S. and Haug, A.: Production of carbohydrates by the marine diatom *Chaetoceros affinis* var. *willei* (Gran) Hustedt. I. Effect of the concentration of nutrients in the culture medium. J. Exp. Mar. Biol. Ecol., 9, 125-136 (1972).

10. Holm-Hansen, O. *et al.*: Quantitative micro-determination of lipid carbon in microorganisms. Analyt. Biochem., 19, 561-568 (1967).

11. Mann, J.E. and Myers, J.: On pigments, growth and photosynthesis of *Phaeodactylum tricornutum*. J. Phycol., 4, 349-355 (1968).

12. Goldman, J.C.: Outdoor algal mass cultures -- II. Photosynthetic yield limitations. Water Research, 13, 119-136 (1979).

13. Goldman, J.C.: Outdoor algal mass cultures -- I. Applications. Water Research, 13, 1-19 (1979).

14. Goldman, J.C.: Physiological aspects in algal mass cultures. In Shelef, G. and Soeder, C.J. (eds.) Algae Biomass. Production and Use, Elsevier/North Holland Biomedical Press, Amsterdam, 1980, pp. 343-359.

15. Shelef, G. *et al.*: Algal mass production as an integral part of a wastewater treatment and reclamation system. In Shelef, G. and Soeder, C.J. (eds.) Algae Biomass. Production and Use, Elsevier/North Holland Biomedical Press, Amsterdam, 1980, pp. 163-189.

16. Ansell, H.D. *et al.*: Studies on the mass culture of *Phaeodactylum*. II. The growth of *Phaeodactylum* and other species in outdoor tanks. Limnol. Oceanogr., 8, 184-206 (1963).

17. Raymond, L.: Initial investigations of a shallow-layer algal production system. Rept. to Hawaii Natural Energy Inst. and the Dept. of Planning and Economic Development, The State of Hawaii, 27 pp. (1977).

18. Lewin, J.C., Lewin, R.A., and Philpott, D.E.: Observations on *Phaeodactylum tricornutum*. J. Gen. Microbiol., 18, 418-426 (1958).

19. Parsons, T.R., Stephens, K., and Strickland, J.D.H.: On the chemical composition of eleven species of marine phytoplankton. J. Fish. Res. Bd. Canada, 18, 1001-1016 (1961).

20. Platt, T. and Irwin, B.: Caloric content of phytoplankton. Limnol. Oceanogr., 18, 306-310 (1973).

ALGAL RESEARCH AT SERI: A BASIC RESEARCH ON PHOTOBIOLOGICAL PRODUCTION OF FUELS AND CHEMICALS BY MICROALGAE

STEPHEN LIEN

SOLAR ENERGY RESEARCH INSTITUTE (SERI)*

GOLDEN, COLORADO 80401, U.S.A.

*SERI is a Division of MRI operated for the U.S. Department of Energy under Contract EG-77-C-01-4042. The author also acknowledges the vital support for portions of this research from Office of Energy Research, the U.S. Department of Energy under OER field task proposal No. 006-80.

I. GENERAL BACKGROUND

The most outstanding feature of the oxygenic photosynthetic organisms (higher plants, eucaryotic algae, and cyanobacteria) is their ability to utilize water molecules as the source of reductant for their biosynthetic processes. As a result, they are true producers of energy, capable of a net conversion and storage of solar radiant energy into the chemical free energy of their biosynthetic products. The algal research project at SERI is a long-term basic research effort attempting to adapt and to modify (by physiological, biochemical, and genetic means) the oxygenic photosynthesis and associated cellular metabolic processes of the eucaryotic algae to produce fuels and chemicals. The basic principle of photobiological fuel and chemical production linked to the oxygenic photosynthesis is illustrated in Fig. 1.

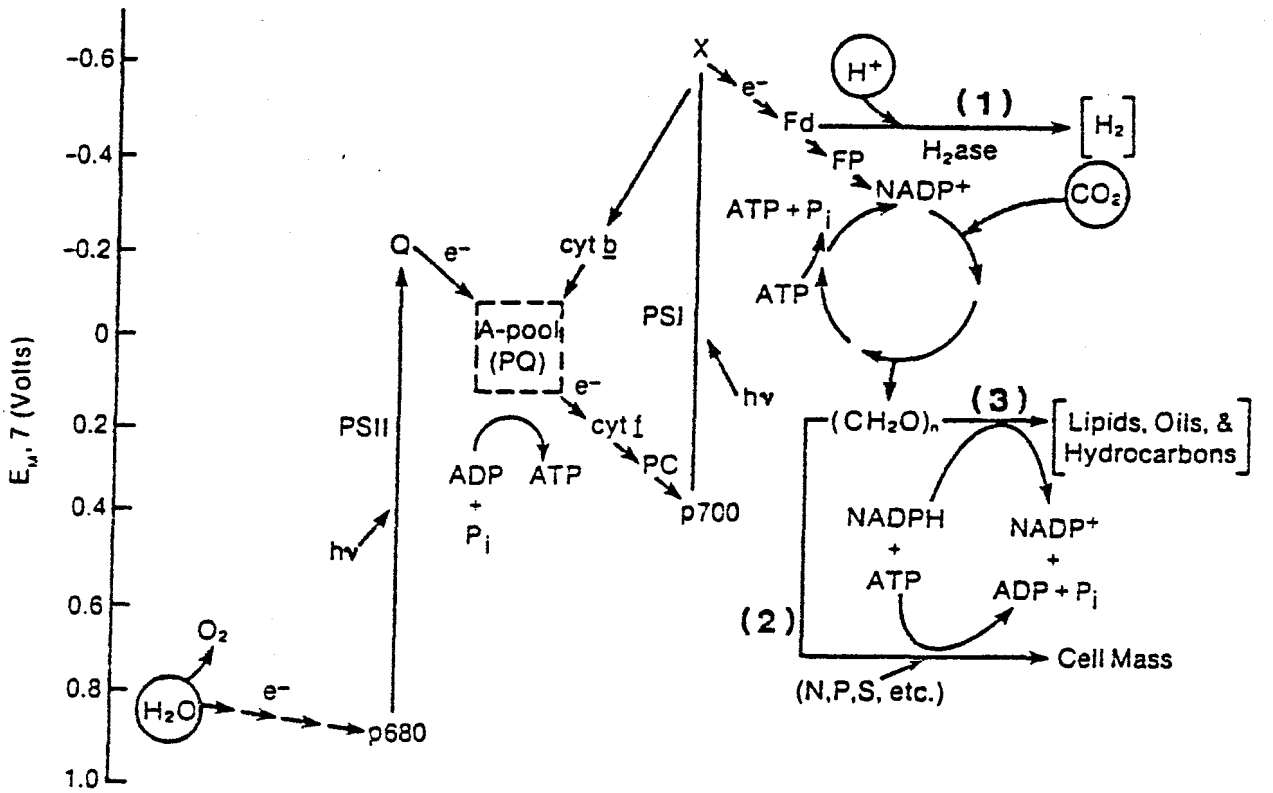


Figure 1. Oxygenic Photosynthesis for Fuel Production

In the oxygenic photosynthetic organisms, a unique light-driven redox reaction (which is catalyzed by a chlorophyll-containing, enzyme-pigment macromolecular complex, known as PHOTOSYSTEM II or PSII, located in the photosynthetic membrane systems of these organisms) extracts electrons from water molecules and liberates molecular oxygen. A second light-driven redox reaction catalyzed by another chlorophyll-containing, macromolecular complex of enzymes and pigments (known as PHOTOSYSTEM I or PSI) further activates these electrons generated by the water-splitting reactions of PSII to a redox potential which is nearly 0.2 V more negative (i.e., having a stronger reducing power) than that of the H_2/H^+ redox couple at pH 7. These highly energetic electrons (reductants) are stabilized in the form of a reduced, low-potential redox carrier molecule, such as ferredoxin (Fd, a non-heme, iron-sulfur protein). In the presence of an appropriate hydrogen catalyst (e.g., hydrogenase), the reduced ferredoxin can donate its electron to the hydrogen ions (H^+) yielding molecular hydrogen (H_2), a gaseous fuel [Fig. 1, reaction (1)]. However, under normal photosynthesis, the low potential reductants are utilized to generate a reduced nucleotide (NADPH), an universal physiological redox carrier, which is used for the reductive fixation of carbon dioxide to carbohydrates. The low-potential photosynthetic reductants (such as ferredoxin or NADPH) also participate directly or indirectly in many reductive biosynthetic processes leading to the formation of more cell mass as the final product [Fig. 1, reaction (2)]. Under special conditions, some species of algae can be induced to channel a large fraction of their photosynthetically fixed carbon into various biosynthetic pathways leading to lipid biosynthesis [Fig. 1, reaction (3)]. Oils and hydrocarbons may accumulate. In some cases, they may constitute a large fraction of the total algal mass. These oleaginous

algae may lend themselves to future adaptation and development as efficient solar energy to liquid-fuel transducers.

II. PROJECT OUTLINES AND PRELIMINARY RESULTS:

Based on the above-mentioned, brief outline of the principle of fuel and chemical production via oxygenic photosynthesis, the algal research project in the Photoconversion Branch at SERI emphasizes on the following two areas of study: (1) hydrogenase system and hydrogen metabolism of green algae; and (2) lipid metabolism and oil/hydrocarbon accumulation in eucaryotic microalgae.

A. Algal Hydrogenase and Hydrogen Production.

Research on the hydrogenase and hydrogen metabolism of algae is directed to obtain basic information needed to resolve the major technical problems (see Fig. 2) associated with algal hydrogen production linked to photobiological oxidation of water.

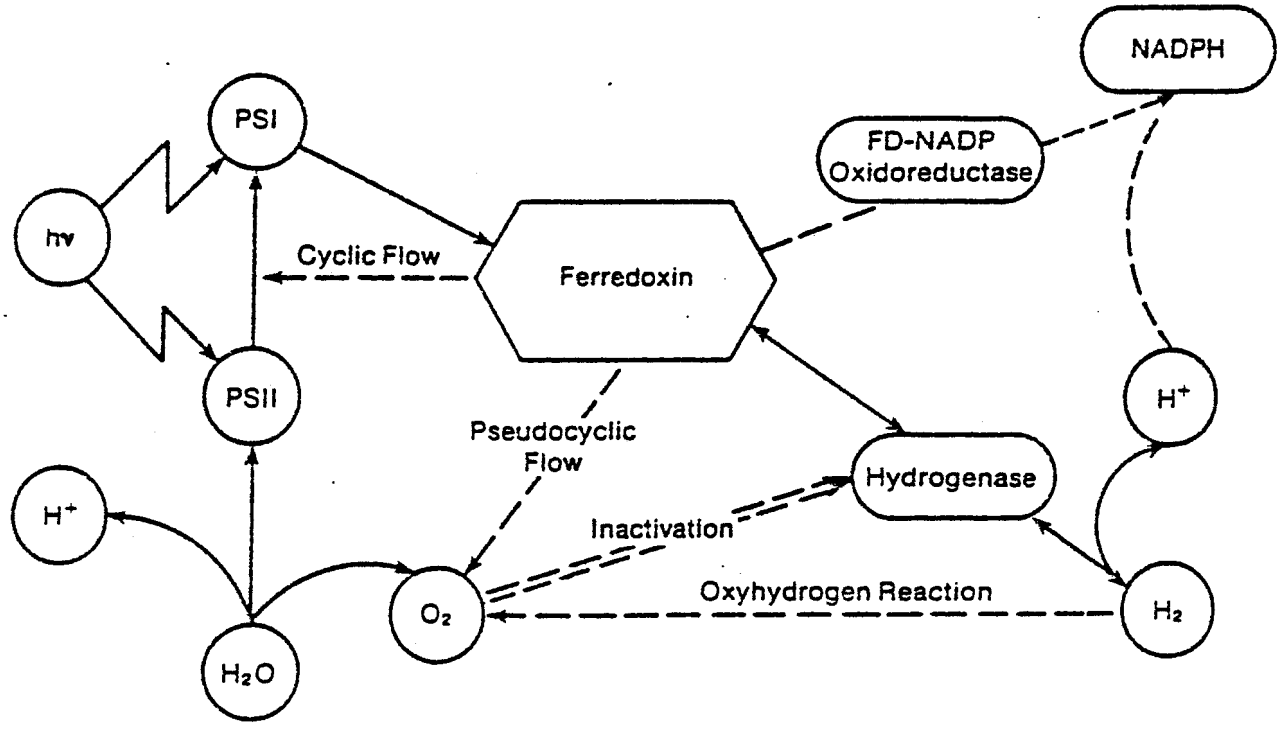


Figure 2. Technical Problem Areas Associated with Photoproduction of H_2 by Algae
The solid arrows indicate reactions leading toward H_2 production, while the broken arrows denote competing side reactions.

Clearly, most of the technical problems arise because whenever water serves as the substrate for hydrogen production, oxygen is liberated as an obligatory by-product. As oxygen concentration builds up, various O_2-induced back reactions (such as the oxy-hydrogen reaction and autooxidation of the photosynthetic redox carriers) are accelerated. These back reactions reduce the yield of H_2 and consequently reduce the net energy efficiency of the system. More critically, in systems using algal hydrogenase as the hydrogen catalyst, oxygen molecules, even in very low concentrations, rapidly deactivate the enzyme. Thus, algal photoproduction of H_2 from water is generally limited to very short durations. In most cases, simultaneous O_2 and H_2 production of eucaryotic algae lasts only a few minutes. To obtain basic information needed to solve the technical problems associated with algal hydrogenase, we have undertaken a systemtic characterization of the hydrogenase system in the unicellular green alga, C. reinhardtii, with special emphasis on: (a) understanding biochemical events leading to the appearance of active algal hydrogenase during anaerobic adaptation in vitro, and (b) characterizing the biochemical and catalytic properties of the enzyme in vitro.

Evidence was obtained indicating that an energy-requiring (or ATP-consuming) step is involved in the process of hydrogenase activation during anaerobic incubation. Recently we observed a very strong stimulatory effect by various anions on the catalytic activity of the algal enzyme in cell-free reactions involving the nonphysiological redox carrier, methyl viologen (MV). Interestingly, we also observed that anions are potent competitive inhibitors whenever the redox reactions catalyzed by the enzyme is mediated via the physiological electron carrier, ferredoxin. Additional data and the technical implication of this anionic effect will be presented.

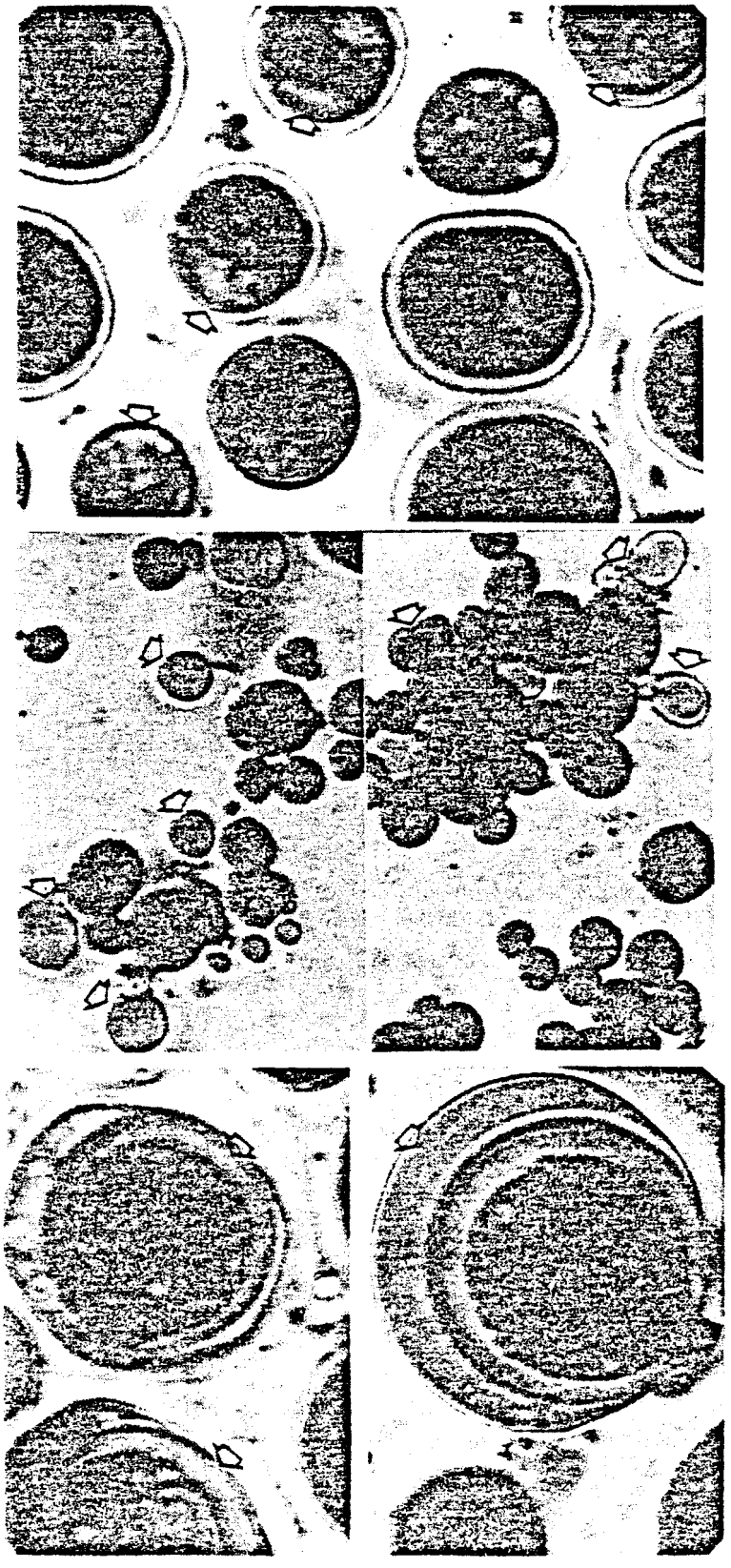
B. Algal Lipid Metabolism and Oil/Hydrogen Accumulation.

The term algal oil and hydrocarbon is defined as that class of carbonaceous, lipoidal compounds which are produced by the algal cells, are not covalently linked to the cellular proteins or carbohydrates, and are characterized by a high content of reduced carbon (such as $-CH_3$, $=CH_2$, or $\equiv CH$) relative to that of the oxygenated carbon (such as $\equiv COH$, $=C=O$ or $-COOH$). Thus, algal oil and hydrocarbon are, in principle, readily extractable and easily separated from other cellular constituents. Because of their high reduced-carbon content, algal oil and hydrocarbons have high caloric value and may be converted into fuels without a large net input of additional chemical energy.

For the analysis of lipid metabolism and oil/hydrocarbon accumulation in algae, we have focused our attention, mainly, on unicellular, eucaryotic algal species of fresh-water and soil origin. The major emphases here are: (1) to understand the biosynthetic pathways involved in the production of algal oils and lipids; and (2) to analyze the control mechanisms involved in the regulation of the oleaginous capacity in algal cells. The ultimate goal of this research effort is to obtain the basic and fundamental information necessary for the development of biochemical and genetic manipulations of algal oil and lipid productivity so that oleaginous algal species can be used as efficient solar energy to liquid fuel and chemical transducers.

Recently, we developed highly efficient cytochemical staining techniques for screening and evaluating a large number of algal species for their oleaginous capacity. This staining technique is based on a drastic change in solubility as well as a large spectral shift accompanying the protonation and deprotonation of the dye molecule CI Basic Blue 12. In an acidic aqueous

solution (pH <5) the dye is protonated and absorbs strongly in the red spectral region. The blue protonated dye in acidic aqueous solution is not readily extracted into the n-nonanl. At neutral to mildly alkaline pH, the dye readily partitions into the lipoidal phase as the deprotonated free base that absorbs strongly in the blue spectral region and thereby has a yellowish-orange color. When samples of algae are pulse stained with the dye "CI Basic Blue 12" under appropriate conditions, the intracellular oil droplets of the cells can be readily observed as yellowish-orange structures, while more polar cellular constituents are stained deep blue (see Fig. 3). Using this technique, a large number of algal species grown under diverse nutritional conditions can be readily screened for their ability to produce and accumulate oils. Our initial screening identified the following species as good potential oil or hydrocarbon producers: Neochloris oleoabundans, N. pseudostigmata, N. texensis, and Chlorocroccum oleofaciens. The effect of nutritional physiological parameters on relative rate of increase of algal mass and oil content of these organisms is currently being investigated.



A. Old cells of C. oleofaciens accumulate copious amount of ois (arrows).

B. Oil droplets (arrows) are readily released from notrogen-starved cells of N. oleoabundans.

C. The cells of N. pseudostigmata produce oil of low apparent viscosity. Under moderate mechanical pressure the intracytoplasmic oil (arrows) accumulates in the space between the partially raptured cytoplasmic membrane and the intact cell wall.

FIG.3 EXAMPLES OF OLEAGINOUS ALGAE

DR. JOHN BENEMANN

Ecoenergetics, Inc.

Paper Not Available at Time of Printing

ASSESSMENT OF BLUE-GREEN ALGAE IN SUBSTANTIALLY REDUCING NITROGEN FERTILIZER REQUIREMENTS FOR BIOMASS FUEL CROPS

D. B. Anderson[1], P. M. Molton[1] and B. Metting[2]
Pacific Northwest Laboratory, Richland, WA 99352[1]
and
R&A Plant/Soil, Inc., Pasco, WA 99301[2]

ABSTRACT

Laboratory, mass culture and field studies are being undertaken in order to assess the potential of using blue-green algae (cyanobacteria) as nitrogen biofertilizers on irrigated ground. Of seven candidate strains, two were chosen for application to replicated field plots sown to field corn and the basis of laboratory-scale soil tray experiments at Battelle PNL and ease of semi-continuous 8,000 ℓ culture at R&A Plant/Soil, Inc. Chosen were Anabaena BM-165, isolated from a local soil and Tolypothrix tenuis, imported from India. Using the acetylene reduction method, Anabaena is estimated from laboratory soil experiments to be able to fix from 30-62 kg N/ha/year, and has been mass cultured to a density of 1527 mg dry wt/ℓ. T. tenuis is estimated from laboratory experiments to be able to fix from 27-65 kg N/ha/year, and has been mass cultured to a density of 1630 mg dry wt/ℓ.

INTRODUCTION

Blue-green algae (cyanobacteria) are photosynthetic, O_2-evolving prokaryotic microorganisms, many of which are able to satisfy cellular and metabolic nitrogen needs by fixing N_2 from the air. In addition, certain blue-green algae are able to fix nitrogen under aerobic conditions, an ability not possessed by the majority of other diazotrophic bacteria, due to the presence of specialized N_2-fixing cells known as heterocysts. For further details regarding the ecology and biology of blue-green algae, particularly on soil, see Stewart [1] and Metting [2].

It is well known that N_2-fixing blue-green algae are integral components of cultivated and uncultivated soils [2]. Indeed, free-living blue-green algae have for years been used in India to supply N to rice in flooded soils and, to a lesser extent, to irrigated corn, sugarcane and vegetable crops [3]. While it is well documented that endemic populations of blue-green algae fix from 15-100 kg N/ha/yr on temperate soils [4,5,6], the potential for using mass cultures as a nitrogen supplement or substitute with irrigated crops in temperate zones has not been assessed.

Information regarding mass cultivation of nitrogen-fixing blue-green algae other than that reported by Watanabe [7,8] and Venkataraman [3] is not readily accessible.

OBJECTIVES

The overall goal of the project is to quantify the extent to which the nitrogen requirements of corn can be met using mass cultured blue-green algae. Specific objectives include 1) isolation or other procurement of candidate strains of blue-green algae, 2) selection of the most favorable medium for growth of candidate algae, 3) monitoring growth and nitrogen fixation of candidate algae on soil in short term laboratory experiments, 4) quantifying growth of candidate algae in 8,000 ℓ semi-continuous mass culture, 5) selection of two blue-green algae for field testing on the basis of results gathered from laboratory experiments and mass culture trials, 6) application of mass cultured algae to replicate plots sown to field corn 7) quantification of algal growth, nitrogen fixation, fluctuations in soil N, and yield of corn, and 8) assessment of the potential large-scale impact of bio-fertilization

with blue-green algae on the overall energy budget associated with production of irrigated crops.

METHODS AND RESULTS TO DATE

Procurement of Candidate Blue-Green Algae

Objective 1 was accomplished by assembling algal cultures isolated by Metting [9] (Anabaena BM-165, Nostoc muscorum, and Cylindrospermum sp.), and borrowed from G. S. Venkataraman, Indian Culture Collection of Microalgae (Tolypothrix tenuis, Aulosira fertilissima, Anabaena V-310, and Nostoc v-220). Cultures were maintained on agar slants and in soil-water tubes.

Laboratory Growth Experiments

Experiments were undertaken at Battelle PNL in order to ascertain the best medium in which to attempt mass culture of each alga. Media compared were those of Fogg [10], Gorham [11], Kratz & Meyers [12], and Watanabe [3,7]. Two hundred and fifty ml cultures of each of the seven candidate algae were maintained for ten days at 30°C under continuous cool white light at 120 rpm in a New Brunswick Model G-27 Psychrotherm Incubator Shaker. Standard inocula were prepared and dry weights obtained as previously described [13]. Experiments were performed in triplicate and repeated twice.

Results showed that Anabaena BM-165 grew best in Gorham's medium, Cylindrospermum sp. in Kratz & Meyers medium, and Anabaena V-310 in Fogg's medium. The other algae grew equally well in two media, as follows; Nostoc muscorum (Gorham's Kratz & Meyers), Aulosira fertilissima (Fogg's, Gorham's) Tolypthrix tenuis (Fogg's, Gorham's), and Nostoc V-220 (Fogg's, Gorham's).

Soil Tray Experiments

Experiments at Battelle PNL were undertaken using 25 x 50 cm thick-walled pyrex baking dishes inoculated with late log phase algal cultures homogenized in 15 ml ground glass tissue grinders and diluted to approximate a field application rate of 65 ℓ/ha. These experiments were undertaken for two reasons; 1) in order that another strong criterion, i.e., growth on local soil, be tested prior to choosing two blue-green algae for field studies, and 2) in order to acquire familiarity with the mechanics of a) estimating N_2-fixation by the acetylene reduction technique [14], b) estimating growth of algal populations by a modified most-probable-number method [15], and c) monitoring fluxes in soil NO_3^-, NH_4^+ and crop-available N [16].

Soil was collected from the field site, air-dried, sieved and layered into the dishes to a depth of 5 cm (about 5,000 g). The soil was brought to 125% of field capacity with deionized water, inoculated with algae using a hand held plant sprayer, and incubated for 26 days at 30°C under cool white light on a 16:8 (light:dark) photoregime. An uninoculated tray was prepared in order to serve as a control with which to study the native algal flora. Each alga was tested at least twice with visible growth of added algae apparent within ten days in all cases. As inoculation with Anabaena BM-165 and Tolypothrix tenuis always resulted in even growth on the surface, these two algae were chosen for detailed measurement of acetylene reduction and population growth.

Ethylene production was quantitated with a Perkin-Elmer gas chromatograph by flame-ionization detection in one ml samples taken from soil crusts incubated in 10% C_2H_2 for one hr at 30°C under cool white light in 15 ml culture tubes sealed with serum stoppers. Crusts were collected by inserting a one cm diameter cork borer into the soil, pushing the core through the borer and removing the upper mm with a razor blade. Values reported in Table 1 are averages of three replicates. In no case did crusts incubated without C_2H_2 demonstrate values greater than 0.32 ppm C_2H_4, and

was not detectable in most instances. No significant statistical correlations with indexes of soil nitrogen were noted, as was expected in view of the relatively short time period and quantities of soil necessary to affect accurate measurements.

Results of the soil tray experiments listed in Table 1 show that both the selected strains competed favorably with the native algae and that significantly different rates of acetylene reduction were measureable. However the extrapolation made to kg/ha/year of fixed N should only be regarded as an indication of potential at this point. It is well known that moisture, time of day, the metabolic status and age of the algal population, and edaphic biological and chemical fluctuations all influence acetylene reduction [1]; therefore variation in the values listed are not surprising but rather expected.

Table 1. C_2H_2-reduction by Algal Crusts and Extrapolation to Large-Scale N_2-fixation for Anabaena BM-165, Tolypothrix tenuis and Native Algae.

Algal Flora	Age (days)	ppm C_2H_4/44mm^2/hr	kg/ha/year	MPN†
Anabaena BM-165	5	6.80	47-62	$2.0 \times 10^{5}_{8}$
	12	5.71	39-52	2.4×10^{9}
	19	4.40	30-52	1.0×10^{9}
	26	3.83	26-35	5.2×10^{6}
Tolypothrix tenuis	5	7.22	49-65	1.9×10^{9}
	12	6.86	47-62	1.1×10^{9}
	19	6.13	42-55	6.7×10^{9}
	26	3.99	27-36	4.8×10^{4}
Native	5	0.79	5-7	2.0×10^{5}
	12	-	-	2.8×10^{5}
	19	-	-	7.7×10^{7}
	26	3.09	21-28	5.1×10^{7}

*Range of values represent limits of theoretical ratios of acetylene reduction to nitrogen fixation. Where one year is equal to a 100 day season of 10 hour days.
†Most-Probable-Number of blue-green algal particles per gram air-dry soil.

Mass Culture

Semi-continuous 8,000 ℓ mass culture experiments at R&A Plant/Soil, Inc., were undertaken to determine ease of preparation of inocula for field tests. Each of the seven candidate algae were mass cultured at least twice in Gorham's medium. Only Anabaena BM-165 and Tolypothrix tenuis were successfully cultured to a maximum density of at least 1000 mg/ℓ and less than 20% contamination (as assessed by cell counts) with unwanted algae and protozoa. Average values for final density, specific growth rate and mean doubling time are included in Table 2.

Table 2. Growth Parameters for Mass Cultured Algae.

Alga	Specific growth rate μ	Mean doubling time (hr)	Maximum density (mg/ℓ)
Anabaena BM-165	.76	24	1700
Tolypothrix tenuis	.26	43	1630

Field Studies

On the basis of soil tray and mass culture experiments, Anabaena BM-165 and Tolypothrix tenuis were chosen as the two algae to be used in the field work.

Fifteen 15 m^2 plots were established, including three each inoculated with one or the other selected alga, three fertilized with 50 kg N (as NH_4NO_3)/ha, three with 100 kg N/ha and three without algae or fertilizer. In Table 3 are summarized those events relevant to field work through June 2.

Table 3. Chronology of Events Associated with Field Studies of Biofertilization with Blue-Green Algae (1981).

April 17	Alfalfa sprayed with Roundup^R (isopropylamine salt of N-phosphomethyl glycine)
May 1-3	Seedbed prepared.
May 20	Northrup-King PX-14 field corn sown.
May 27-28	Corn emerges.
May 29	Anabaena BM-165 inoculated onto plots at about 380 ℓ/ha of a 1527 mg/ℓ mass culture.
June 2	Tolypothrix tenuis inoculated onto plots at about 380 ℓ/ha of a 1630 mg/ℓ mass culture.

Literature Cited

1. Stewart, W. D. P. Systems involving blue-green algae (cyanobacteria). in Methods for Evaluating Biological Nitrogen Fixation. F. J. Bergersen (ed.). Wiley & Sons, New York (1980), pp. 583-635.

2. Metting. B. The systematics and ecology of soil algae. Botanical Review 47 (2), in press (1981).

3. Venkataraman, G. S. Algal Biofertilizers and Rice Cultivation. Today and Tomorrow's Printers and Publishers, New Dehli (1972).

4. Witty, J. F. Algal Nitrogen Fixation on Solid Surfaces and Temperate Agricultural Soils. Ph.D. Dissertation, Univ. London (1974).

5. Dart, P. H. and J. M. Day. Non-symbiotic nitrogen fixation in soil. in Soil Microbiology. N. Walker (ed.). Wiley & Sons, New York (1975), pp. 225-252.

6. Henriksson, E. A., L. E. Henrikssen and E. J. DaSilva. A comparison of nitrogen fixation by algae of temperate and tropical soils. in Nitrogen Fixation by Free-Living Microorganisms. W. D. P. Stewart (ed.) Cambridge Univ. Press, Cambridge (1975), pp. 199-206.

7. Watanabe, A. On the mass-culturing of the nitrogen-fixing blue-green alga Tolypothrix tenuis. J. Gen. Microbiol. 5, 85-91 (1959).

8. Watanabe, A. Collection and cultivation of nitrogen-fixing blue-green algae and their effect on the growth and crop yield of rice plants. Pro. Symp. Algology, New Dehli (1960).

9. Metting. B. A Comparative Study of Algal Communities on Cultivated and Uncultivated Portions of a Schumacher Silt Loam. Ph.D. Dissertation, Washington State Univ., Pullman (1979).

10. G. S. Venkataraman, personal communication.

11. Gorham, P. R., J. S. McLachlan, U. T. Hammer and W. K. Kim. Isolation and culture of toxic strains of Anabaena flos-aquae (Lyngb.) de Breb. Verh. Int. Ver. Limnol. 15, 796-803 (1964).

12. Starr, R. C. The culture collection of algae at the University of Texas at Austin. J. Phycol. 14, supplement (1979).

13. Metting, B. New species of green microalgae (Chlorophycophyta) from an Eastern Washington silt loam. Phycologia 19, 296-306 (1980).

14. Turner, G. L. and A. H. Gibson. Measurement of nitrogen fixation by indirect means. in Methods for Evaluating Biological Nitrogen Fixation. F. J. Bergersen (ed.). Wiley & Sons, New York (1980), pp. 111-138.

15. Zimmerman, W., B. Metting and W. R. Rayburn. The occureence of blue-green algae in silt loams of Whitman County, Washington. Soil Science 130, 11-18 (1980).

16. Bremner, J. M. Nitrogen availability indexes. in Methods of Soil Analysis. C. A. Black (ed.). Amer. Soc. Agron., Madison (1965), pp. 1324-1345.

WATER HYACINTH WASTEWATER TREATMENT SYSTEM

C. A. Lee, M.S.
Assistant Project Manager
WED Enterprises
Glendale, California 91201

T. McKim, M.S.
Project Engineer
Reedy Creek Utility Company
Lake Buena Vista, Florida 32830

ABSTRACT

A prototype water hyacinth wastewater treatment system has been in operation at Walt Disney World, Florida, since July of 1979. During this period, this system has been operating with three objectives: 1) to demonstrate an unconventional energy conservative wastewater treatment system; 2) to determine the ability of water hyacinths to treat primary effluent to secondary treatment standards in a flow-through system; and, 3) to try to maximize production of a potential energy feedstock while treating wastewater. Calculations indicate the water hyacinth system requires less than 50% of the energy needed to run a comparably sized conventional secondary treatment system. The effluent from the water hyacinth system demonstrates 80-90% removal of total suspended solids and B.O.D., meeting secondary treatment standards when coupled with primary treatment. In the first year of operation, the amount of water hyacinths harvested from the 3/4 acre system was approximately equivalent to 21 dry tons/acre-year (47.8 dry metric tons/hectare-year). The effects of varying harvest routines and detention times are being investigated to try to optimize wastewater treatment and biomass production. Studies on nutrient removal as well as biomass conversion to methane gas are also being performed.

WATER HYACINTH WASTEWATER

TREATMENT SYSTEM

C. A. Lee, M.S.
Assistant Project Manager
WED Enterprises
Glendale, California 91201

T. McKim, M.S.
Project Engineer
Reedy Creek Utility Company
Lake Buena Vista, Florida 32830

INTRODUCTION

The Water Hyacinth Wastewater Treatment System at Walt Disney World has been in operation since July 1979. The project is operating with these objectives.

1) To determine an unconventional energy conservative wastewater treatment system.
2) To determine the ability of water hyacinths to treat wastewater to both secondary and tertiary treatment standards in a flow through system.
3) To maximize production of a potential energy feedstock while treating wastewater.

In Phase II of this project, part of which has already begun, we will add two more objectives to our operational goals, they are:

4) to demonstrate and optimize the bioconversion of water hyacinths to methane.
5) to treat wastewater to tertiary treatment standards.

IGT through sponsorship with GRI has performed the baseline studies on methane production from Walt Disney World water hyacinths, and operation of two 50-liter digestors, designed by IGT, is already underway at Walt Disney World.

The SERI Contract will expand the system by intergrating three (3) additional channels with the same dimensions, materials and design of the orginal channels.

The purpose of the addition will allow for more rapid evaluation and optimization of the systems parameters, maximize plant productivity, and increase the biomass production capability.

The objective will be to maximize plant productivity without adversly affecting the systems capacity to treat wastewater effectively.

With the SERI addition to the system, the invest igations will include evaluation of the following variables on water quality and plant productivity:

1) Increased detention times
2) Use of a protective cover during winter months
3) Use of mechanical aeration
4) Varying harvesting routines
5) Addition of nutrients and/or metals for maximizing phosphorus removal and maximizing growth.

DESIGN OF SYSTEM - PROJECT COMPONENTS

The system can be broken down into several components: 1) the Production/Treatment channels; 2) the Harvesting System; 3) the Compost System. Special studies are also being performed.

Production/Treatment Channel:

Components of the production system include three 1/4 acre concrete channels, the system piping, utility tie-ins, hydraulic control and metering devices, and pumping stations.
The walls of the channels are constructed of reinforced concrete blocks on a cast-in-place, reinforced concrete foundation. The channels (29' x 360') are lined with 20-mil PVC tacked to the top of the channel walls with lumber. PVC booms are tied off to cleats along the top of the channel walls every sixty-feet and act as a corral preventing the hyacinths from packing together at one end. See Figure #1. The water level in the channels is adjustable, and can be maintained at depths of 14 to 36 inches. The influent gate valves incorporate the capability for each channel to be operated independently and at varied depth. The system is currently treating 63,000 gpd but the system is hydraulically designed to handle flows up to 200,000 gpd. The influent flow is split, with 25,000 gpd to Channel 1, 21,000 gpd to Channel 2 and 17,000 gpd to Channel 3.
Two submersible pumps, one in the effluent channel of the primary clarifier and the other in a filter pump wetwell of the existing RCID Wastewater Treatment Plant can provide the channels with primary and/or secondary effluent. The channels are also interconnected hydraulically to provide a variety of experimental modes of operation (See Figure #2).

FIGURE 1
SYSTEM CONFIGURATION

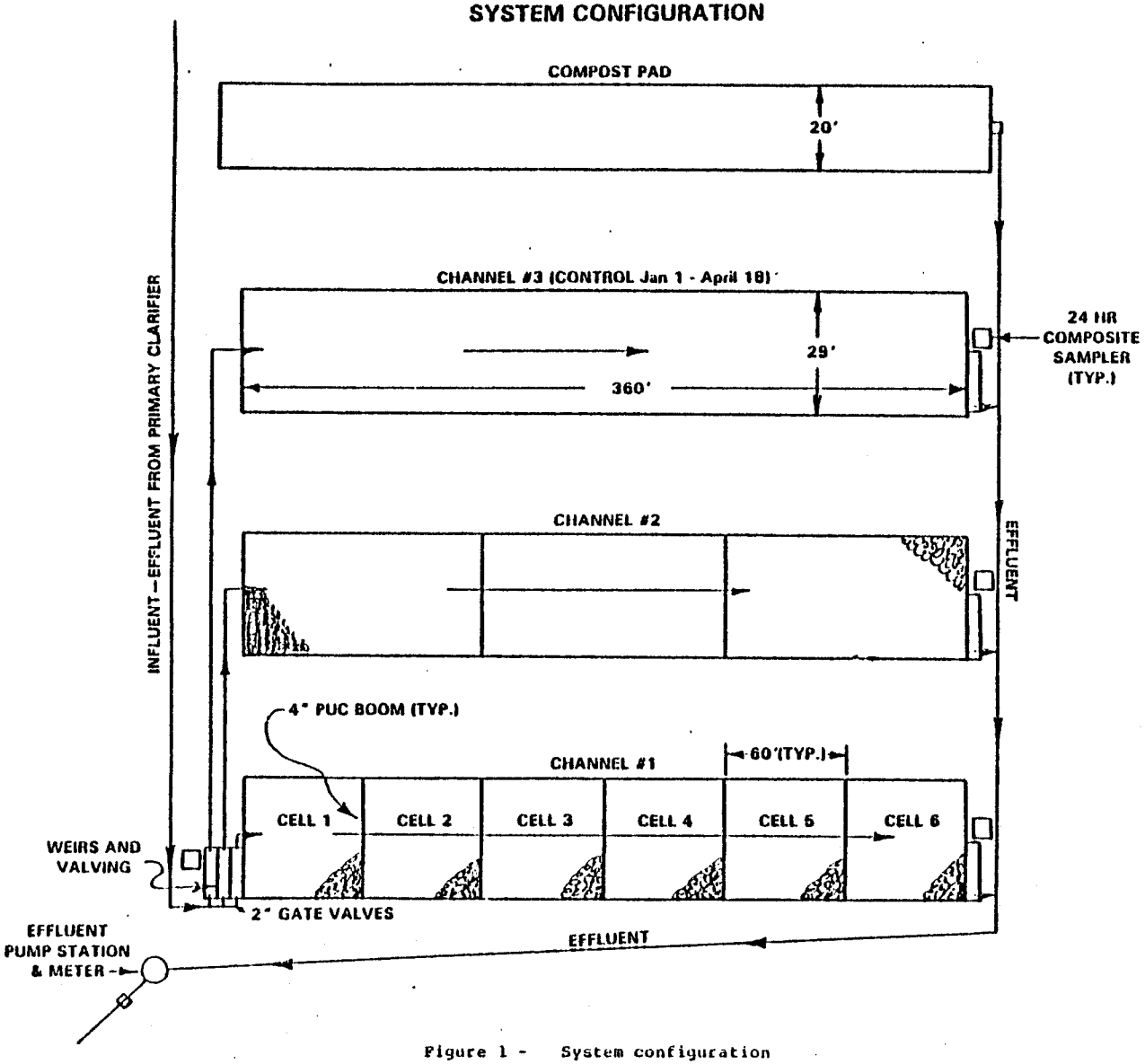


Figure 1 - System configuration

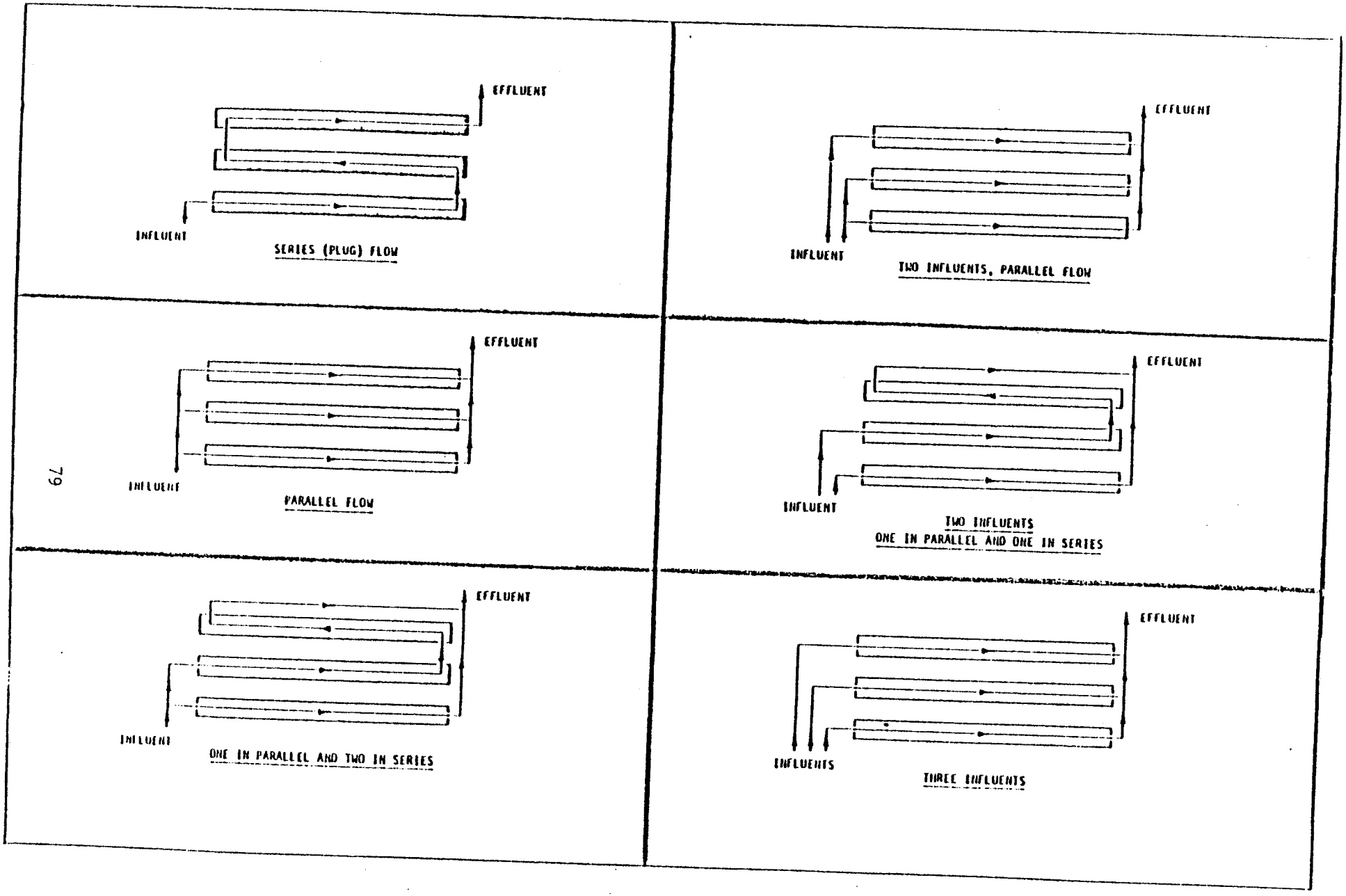


Figure 2 - Possible operational flow modes

Harvesting

The equipment used in harvesting include: 1) a front end loader; 2) a double belt conveyer-chopper; and, 3) a forage wagon. All mechanisms in the system are powered hydraulically from the front end loader. The harvesting system is designed with a capacity of 50 tons/hour, far in excess of the system's requirements. Harvesting is accomplished by physically pushing the hyacinths onto the primary conveyor with a long handled hook. At the end of the primary conveyor, a flail chopper coarsely cuts the plants which are then conveyed to the forage wagon. The forage wagon has a live bed for ease of loading and unloading.

The three channels are divided into six cells 60' long by 29' wide, by floating PVC booms. The booms can be used to push the hyacinths into a uniform density, thereby allowing for a density measurement before and after harvesting. This method assumes that a uniform density is achieved through compaction of the hyacinths, however density data collected in this manner indicated this method was somewhat inconsistent. At the present time, another method of density measurement is being used. In this method, one meter square trays are stocked with a target weight of hyacinths. Each week the tray is weighed and once every other week a portion of the cell is harvested to maintain the target restocking weight. The one meter square tray therefore is used as a representation of the growth of the entire cell. Both methods have often demonstrated crop reduction without the benefit of harvesting. It is believed that the water hyacinths under dense conditions (over 4 wet lbs./sq. ft.) start to slough off material and in many cases the crop weight is significantly reduced.

Composting

The composting operation utilizes a windrow system with a compost pad and front end loader. Once the forage wagon is full, it is moved to the compost pad and unloaded by means of the live bed. All hyacinths harvested from one channel are put into a pile. At the beginning of system operations a new compost pile was turned three times/week during the first week and once a week in the following weeks. Measurements on temperature and free moisture indicated that the desired values of 50-60% moisture and 140°-150°F [1] were not reached in the compost piles. It was then decided to stop turning the piles. The unturned piles reached a maximum temperature of 121°F, but dropped to 80°F after two days.

After a 4-6 month period compost piles resemble a potting soil with an 80+% volume reduction requiring little or no operator attention.

Special Studies

Trace metal studies on Walt Disney World water hyacinths have been performed by the University of Arizona Environmental Research Lab. Initial studies indicated that although Walt Disney World water hyacinths were extremely rich in iron, they contained less Ca, Mg, and K than did water hyacinths grown in nutrient solutions. See Table 1. The uptake of these cations may be inhibited by the uptake of NH_4 + [2]. However, tissue studies performed later in the year did not indicate a lack in Ca, Mg, K. Iron has already proven to be a necessary element in preventing chlorosis (yellowing of the plant), [3, 4]. However, Walt Disney World hyacinths appear to take up an abundance of iron. The large amount of iron found in the tissue may be inhibiting growth and additional studies are being made by the University of Arizona. Further studies will be done to determine the ability of trace metal uptake versus the availability of the metals in Walt Disney World primary effluent.IGT through sponsorship with GRI is performing the baseline studies on methane production from WDW water hyacinths (See Figure #3). The initial study indicates an average yield of 3.0 scf of methane/lb. of volatile solids added to the digestor from Walt Disney World hyacinths. Methane studies will continue as two 50-liter digestors are now being operated at WDW in preparation for a larger digestor or Process Development Unit that will eventually utilize all of the water hyacinths harvested from the three channels.

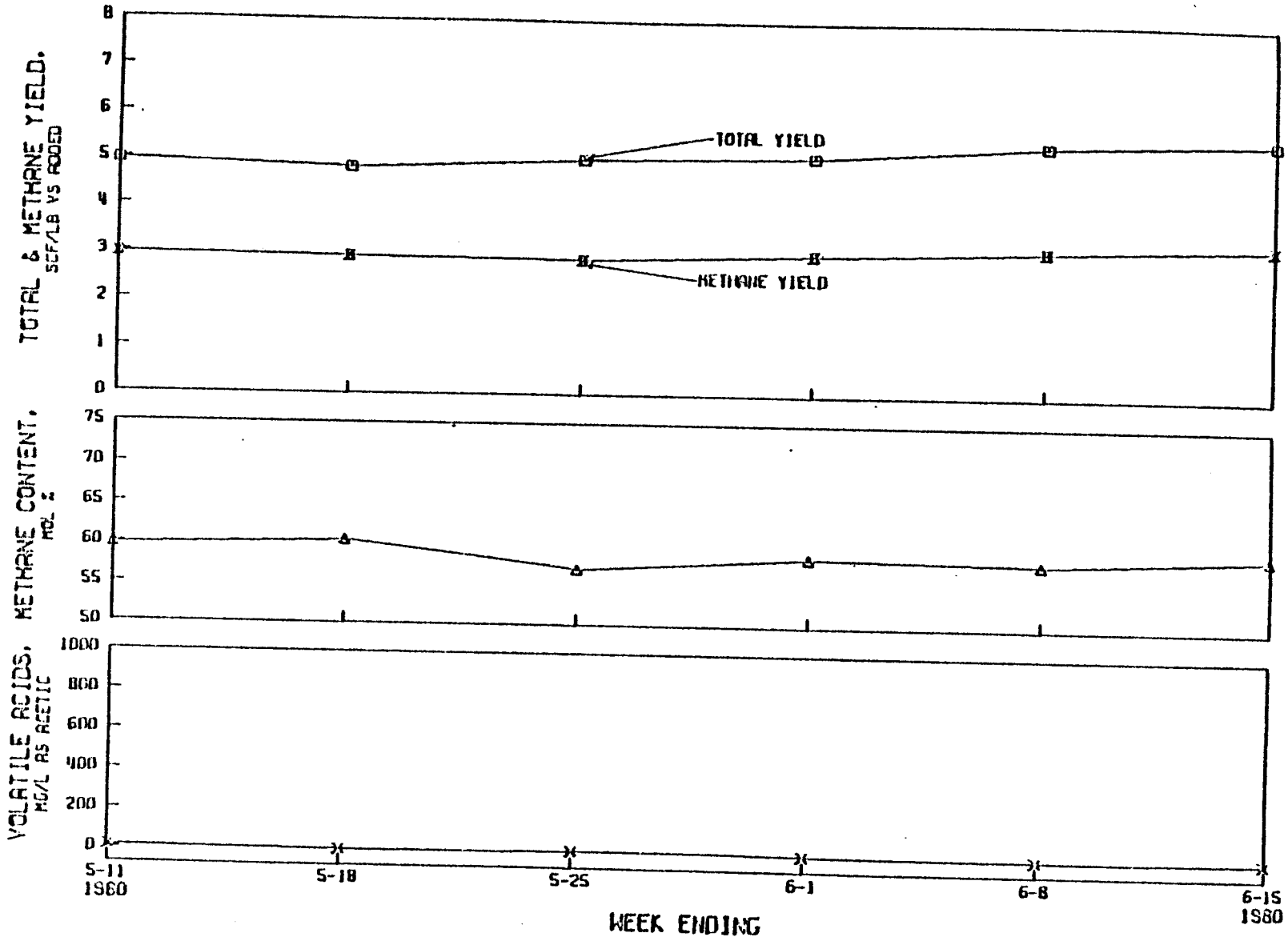
OPERATIONAL MODES

The water hyacinth wastewater treatment system operation began on June 18, 1979 utilizing secondary effluent, and a parallel flow mode. All three channels were seeded with water hyacinths covering approximately 10% of the surface area. Within two months there was essentially 100% plant coverage. The target flow rate was set at 50,000 gpd or approximately 17,000 gpd/channel. On July 13, 1979 at the request of EPA the channels were switched to treat primary effluent. The channel depths were varied with Channel #1 at 14" and Channels #2 and #3 at 36". At these depths, the detention time for Channel #1 was 5.36 days, and 13.8 for Channels #2 and #3. Water quality measurements began on July 2, 1979 and followed the schedule in Table #2.

Table 1 - Tissue analyses of Walt Disney World Water hyacinths from Channels one and two and water hyacinths grown in experimental conditions at the Environmental Research Lab, University of Arizona. Table prepared by ERL, University of Arizona

TISSUE ANALYSIS of WATER HYACINTHS for WDW SAMPLING DATE 1-25-80

	% of dry weight					PPM in dry weight			
	N	P	Ca	K	Mg	Fe	Mn	Cu	B
Channel 1	3.91	.88	.62	2.5	.23	761	23.3	51	35
Channel 2	4.6	1.0	.10	2.3	.25	943	27.5	76	23
ERL Grown	2.5	–	1.3 to 3.0	4.0 to 5	40 to 1.0	20 to 150	60 to 110	15	–



RUN 801, WDW HYACINTH AT STEADY-STATE (L = 0.10, RT = 15)

Figure 3 - Methane production from Walt Disney World Water Hyacinth, Graph prepared by IGT.

WATER HYACINTH WASTEWATER

TREATMENT SYSTEM

TABLE 2

MONITORING

A. Water Quality - Influent & Effluent, Each Channel

1) Daily Tests

a) pH
b) D.O.
c) Water temperature
d) Insolation - Total
e) Rainfall - Total
f) Air temperature and relative humidity
g) Influent and Effluent Flow - Totals

2) Twice per Week

a) TSS
b) TS
c) NH_4^+-N
d) O-PO_4
e) T-PO_4
f) NO_3-N
g) BOD_5
h) TKN

3) Twice per Month

a) Total and Fecal Coliforms
b) Chlorides
c) Alkalinity

B. Bio Mass Production - Each 60' Cell

1) Density (lbs/ft^2) - 1 per week

Table 2 - Monitoring routine

Harvesting began on August 2nd with a harvest schedule set at twice a week for Channels #1 and #2, while Channel #3 was harvested twice a month. At this time, the compaction method of measuring density was utilized to determine the percentage of surface area covered by the crop. A five square foot "cookie cutter" would then segregate water hyacinths for weighing and from this weight and the percentage of covered surface area, the weight of the entire crop was established.

Subsequent to the first water hyacinth review meeting, held on October 19, 1979, including all participants in the project, the operational procedures were changed to the following:

1) Channels #1 and #2 were both maintained at a depth of 14" receiving primary effluent.
2) Channel #1 was harvested twice a week from sequential cells throughout the channel.
3) Channel #2 was harvested once a month and only from the influent end.
4) Channel #3 was used as a control channel maintained at 14" with no water hyacinths.

In January, 1980, the same harvesting schedule was main- tained, however, a seed crop density of two wet lbs./sq. ft. was left in each channel to reseed the harvested area. During this time, PVC booms were used to compact the crop for density measurements. Following the second water hyacinth review meeting, held on April 3, 1980, one major operational change was made:

1) Channel #3 was restocked with water hyacinths and that channel used for density-yield studies. These studies included use of twelve, one-meter square trays made of PVC piping and plastic vexar mesh. These trays were stocked at target densities ranging from 0.5-3.0 lbs./ sq. ft. with duplicates for each target density. These trays were weighed twice a week and the amount over the target density was harvested. At the end of six weeks the optimum density for restocking proved to be 1.5 wet lbs./sq. ft. See Figure #4.

From the above method of measuring growth, it was hoped that the tray method would prove more consistent as a measure of crop density and weight, and the method would be used in the other two channels. At this time it is not clear which method is superior for measuring crop yield.

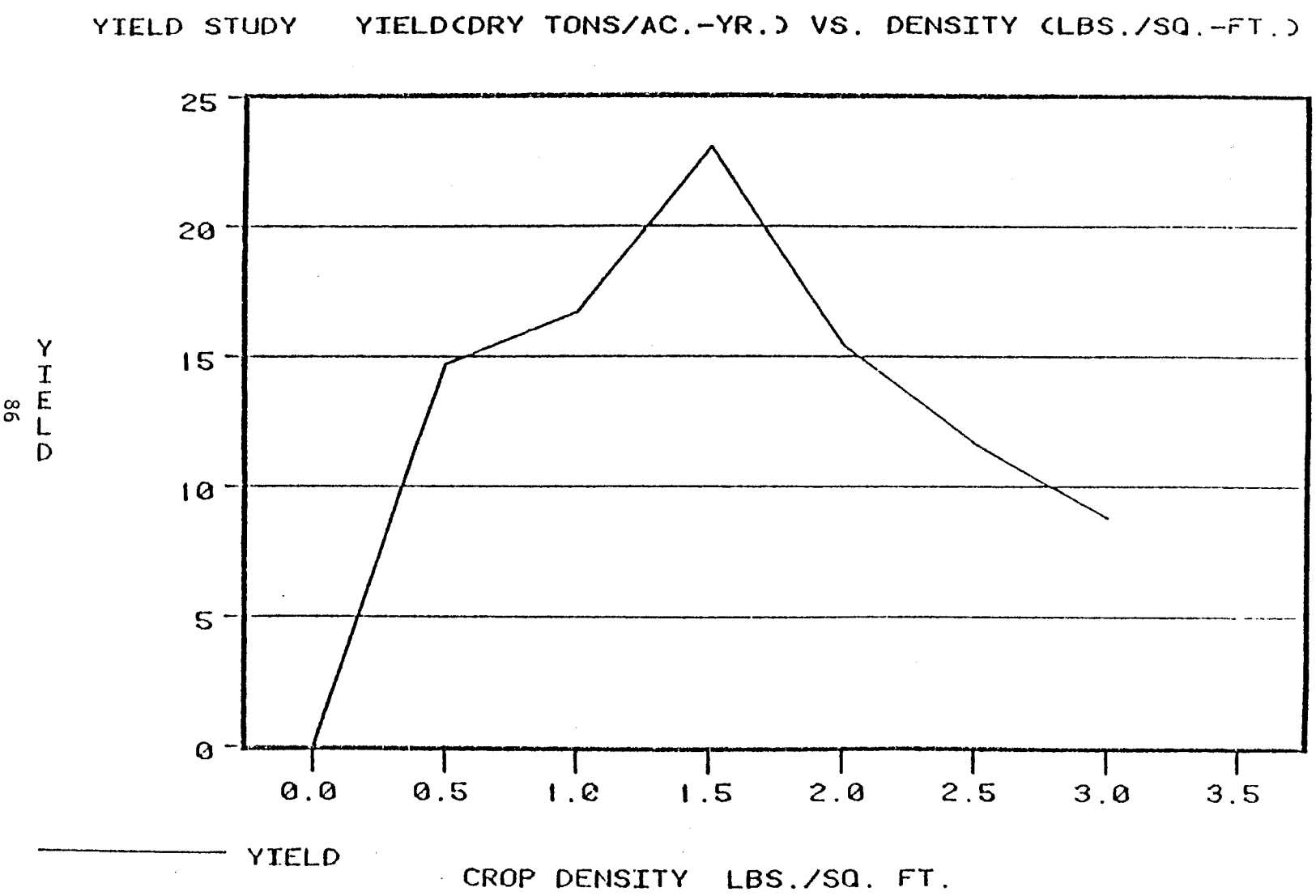


Figure 4 - Yield study shows the optimum density for maximum productivity in wet lbs/sq. ft. yield study continued for six weeks during Sept - Oct 1980.

At the next review meeting, held on November 11, 1980, the following operational decision was made:

1) Increase flow rates in channels one and two by approximately 50% and 25% respectively.

In November, breakdown of the harvesting equipment prevented harvesting during the months of November and December. Cold weather also prevented harvest in January. In spite of these hardships and the increase in flow to channels #1 and #2, the system still managed to remove 90% TSS, however, BOD and nutrient removal did decline.

In March of 1981, two 50-liter digestors were seeded and gas collection and analysis initiated at Walt Disney World. The purpose of this work is to determine the effects on gas quality and quantity from any seasonal changes in the feedstock of water hyacinths and primary wastewater sludge.Currently one digestor is fed primary wastewater sludge and the other is operated on water hyacinths. The digestors are batch fed each day at a rate of 0.15 lbs. volatile solid/cubic ft. of digestor volume. Both are operating on a 15 day detention time. At the time of writing, the digestors have not yet reached steady state.

At the next review meeting held on April 28, 1981, the following operational decisions were made:

1) Channel #3 would be harvested once/week at the influent end only and would maintain a restocking density of 1.5 wet lbs./sq. ft.

2) Channels #1 and #2 would also use a restocking density of 1.5 wet lbs./sq. ft with a harvesting interval of twice/month. Harvest will occur at the influent end only.

3) The flow of 63,000 gpd would be maintained for 3 additional months with the regular harvest routine. At the end of 3 months, the most efficient and effective flow rate for water quality would be evaluated and channels #1 & #2 would then be used in series at that chosen flow rate.

RESULTS AND DISCUSSION

Water Quality

The water hyacinth system has proven to be an effective secondary treatment system. Coupled with primary treatment, the system has met the State of Florida's secondary treatment levels of 90% removal of TSS and BOD. See Figures #5 & #6. The operational changes can be seen in this BOD and TSS profile spanning July 1979 - March 1981. From July, 1979 to October 1979, Channel #2 and #3 were operated at a 36" depth and the water quality data indicate that the deeper depth was not quite as effective as the 14" depth of Channel #1. In October, 1979, Channel #3 was operated as a control channel with no water hyacinths. The graphs indicate a marked increase in suspended solids at this time due mostly to algal generation and a decrease in BOD removal. Also at this time, Channel #2's depth was decreased from 36" to 14" and a corresponding improvement in the effluent of Channel #2 is observed. In May, 1980, when Channel #3 was on line once more stocked with water hyacinths, a marked improvement was observed in the water quality of Channel #3's effluent.

In the summer months of June, July and August, 1980, a decrease in water treatment is observed in both Channels #1 and #2. At this time, many plants were beginning to wither and die. At first, the unhealthiness was attributed to the excessive heat and dryness of an unusual Florida summer, where temperatures reached over 100°F for over three consecutive weeks without any afternoon showers. However, by July, close examination of the plants showed that a moth, Sameodes albiguttales, had infested Channels #1 and #2. In its caterpillar stage, the insect bores into the stolen of the plant cutting off the transport of nutrients from the roots to the leaves. Other insects were also found in the channels including the weevil, Neochetina eichhorniae which eats the leaves in banded patterns approximately 1-2mm thick. [5], another species of weevil N. bruchi and the spider mite, Bryobia praetiosa. The insects appear to attack more heavily when the water hyacinths are stressed, the weevils in cases of density stress [5] and the moth in cases of hot, dry weather stress [6]. The spider mite and weevils are found year round, however, spot harvesting appears to control them very well. It is believed the moth caused the most damage to the channels this summer. Channel #3, restocked in May with water hyacinths, was the only channel not badly attacked.

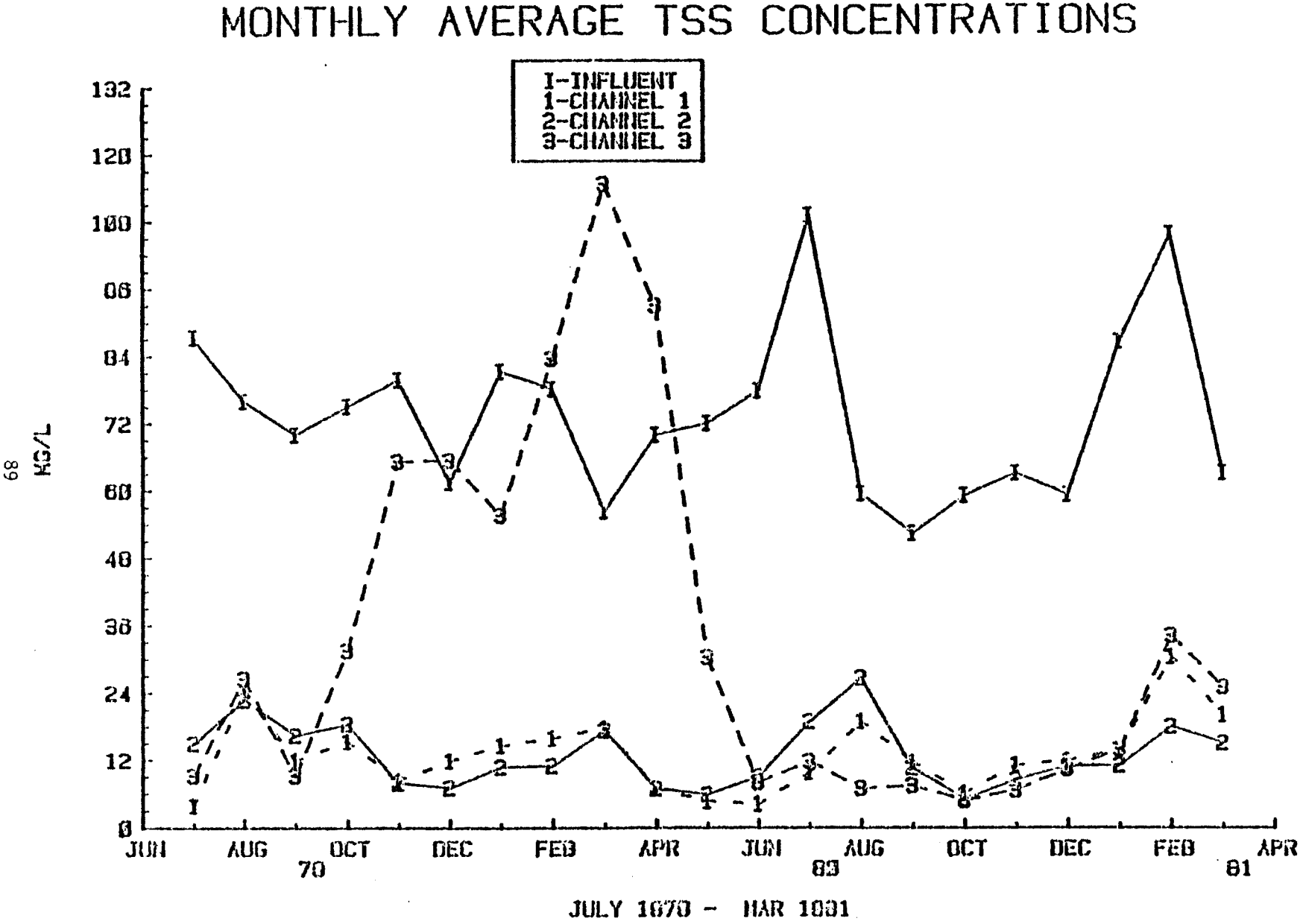


Figure 5 - Monthly average total suspended solids concentrations

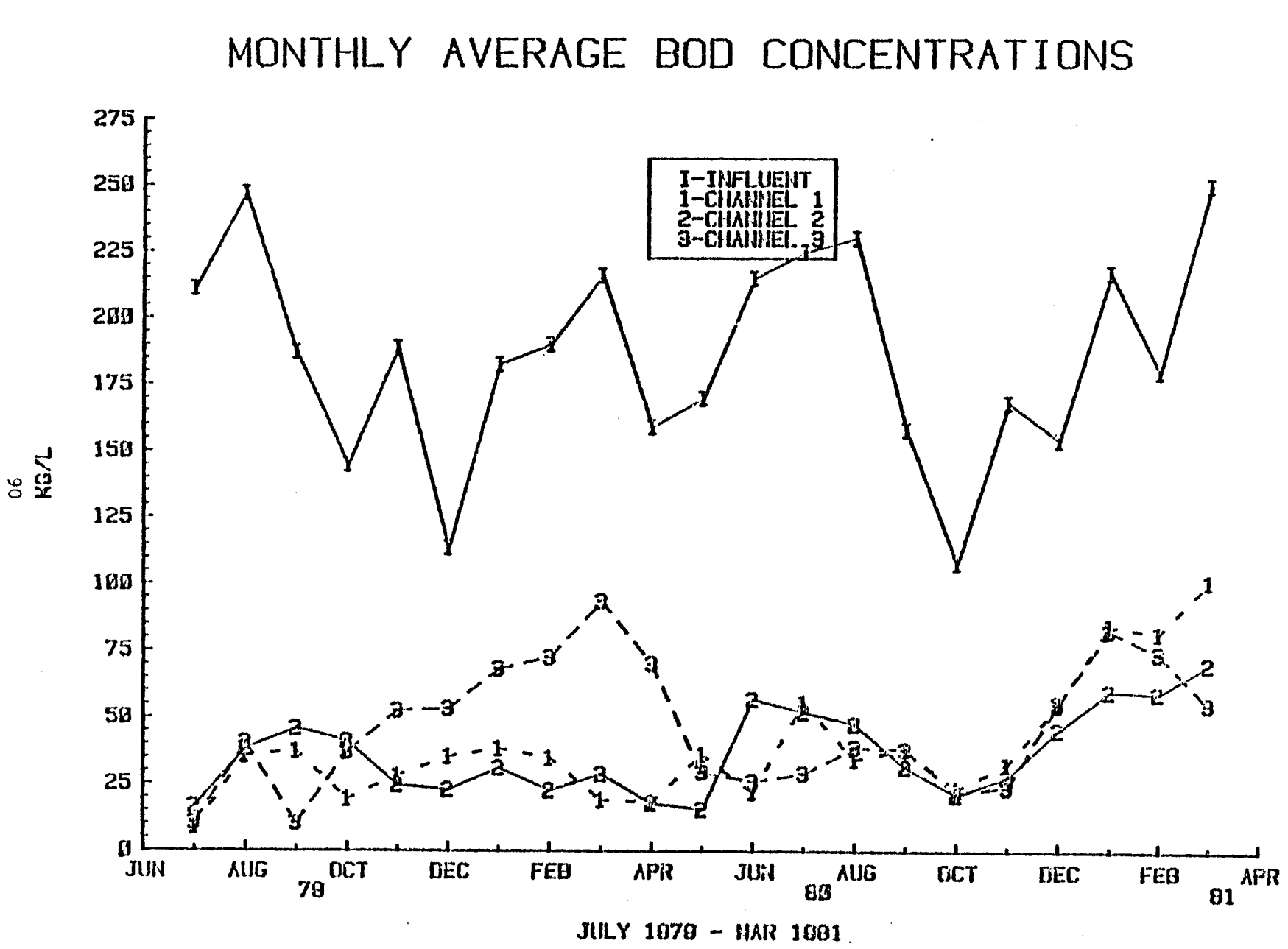


Figure 6 - Monthly average BOD_5 concentrations

In August, all channels were treated with the insecticide Sevin, to prevent further spread of the insects. By the beginning of September, Channels #1 and #2 were beginning to recover and by the end of September they had completely recovered, with BOD and TSS removal returning to higher levels. The application of Sevin was stopped on September 5. Although the hyacinths were able to recover, it was impossible to determine if the insecticide Sevin or if the break in the hot dry spell was responsible for recovery.

The effluent water quality from the channels is very good especially when the effect of evapotranspiration is considered. In October of 1980, a meter and totalizer were installed at the influent end to monitor the volume of water going into the system. The water budget data from the totalizer and the effluent meter indicate a 12-26% evapotranspiration rate and this finding is consistent with previous studies [2]. See figure 7. A water budget is extremely important in evaluating water quality treatment and efforts are being made to monitor the water volume in individual channels rather than measuring the three channels together.

In November and December of 1980, equipment failure prevented harvesting and in January 1981, extremely cold temperatures frost burned much of the crop. Recovery from the frost period was very rapid. The water hyacinths were green within two weeks and recovery was complete within one month. Harvesting resumed in March. In spite of the equipment failure and lack of harvesting, the cold weather, and increased flow to channels one and two, the system still managed to remove 80-90% of the suspended solids. The removal of BOD was not as effective during this period, averaging between 55-77% removal. However at this time it is difficult to assess how much of the reduced performance in BOD is caused by the colder temperatures, the lack of harvesting, or the increase in flow.

Water quality declined in Channel 3 which was restocked in May 1980 with water hyacinths and not harvested until October 1980. During that six month period, water quality improved during the first two months and declined in July and August. Although not as badly attacked as channels 1 and 2, Channel 3 never recovered from the insect infestion and the lack of harvesting appears to be responsible for the continued decline in water quality of Channel 3.

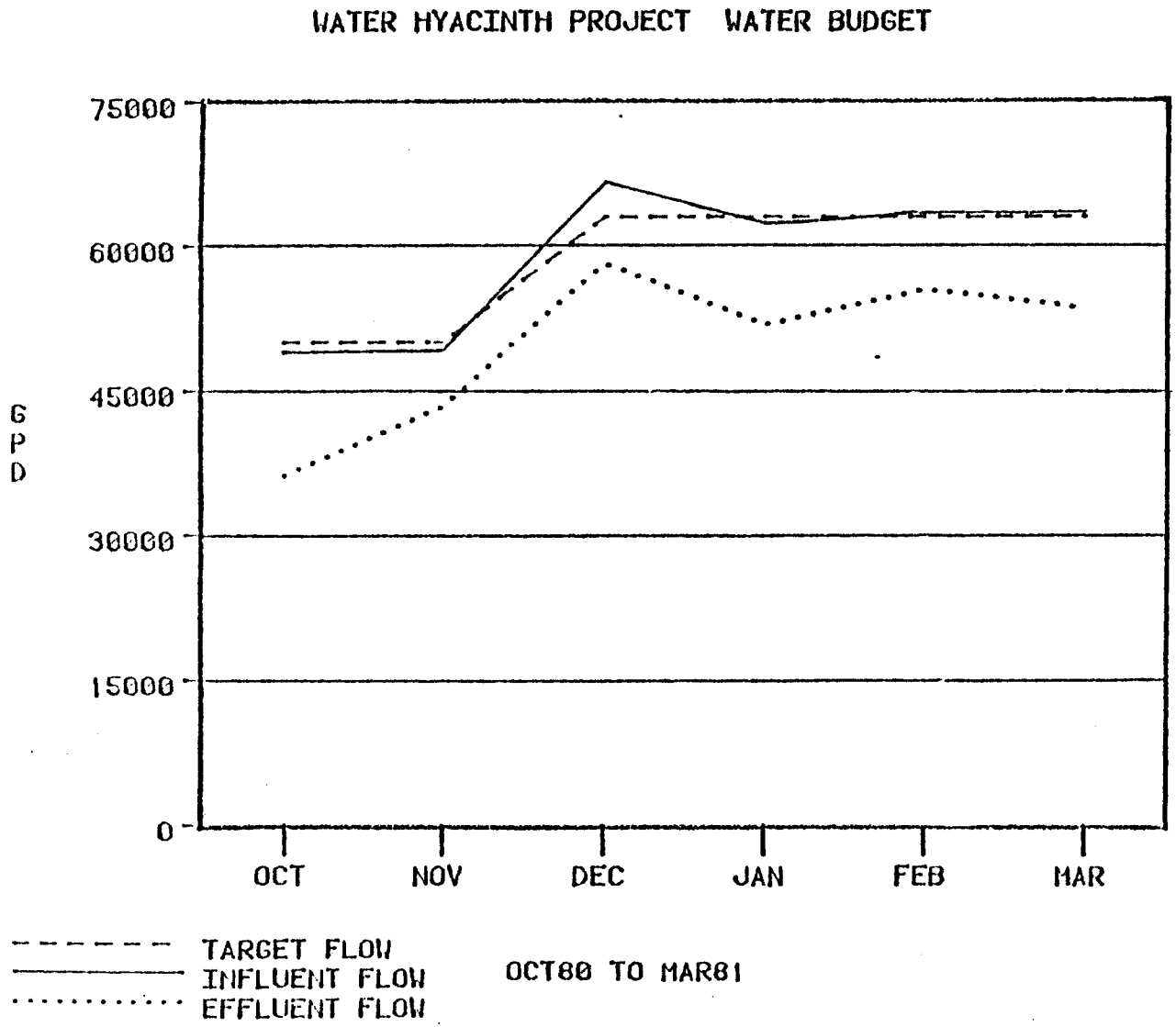


Figure 7 - Water budget data for total system

Nutrient removal in the channels, although fairly consistent throughout the year, does not reach the 90% removal range of TSS or BOD. The channels appear to average approximately 30-50% removal of TKN and 20-40% removal of TPO_4. See figures 8 & 9.

Healthy plant growth is very important for water quality, particularly in nutrient removal as is demonstrated by the graphs of TPO_4 and Total Kjeldahl Nitrogen removal (see figures 8 & 9). The months of January and February in 1980, January and February in 1981, and July and August 1980 are the months showing the least reduction in nutrients. In each case, the water hyacinths were under stress, in January and February by cold temperatures inhibiting growth, and in July and August by the insect infestations which also prevented healthy growth. Harvesting out some of the dead plant material which released nutrients would probably have improved the water quality during the periods of stress, unfortunately due to equipment failure harvesting was not possible during January and February of 1981.

Nutrients appear to be removed at a rate proportional to the loading of the system, even during the winter when the growth rate of the water hyacinths is slower. Bacteria continue to remove nitrogen in the colder months, however, during the warmer months the hyacinths appear more responsible for nitrogen removal. See Figure 10. The bar chart indicates the total amount of nitrogen removed from channel one and the amount removed by the water hyacinths. This chart indicates a trend showing the hyacinths playing a more important role in nitrogen removal during the warmer months.

Phosphorus removal also follows a similar trend to total nitrogen removal in that more phosphorus is removed from the system than can be accounted for in the plants. See figure 11. The amount of nitrogen and phosphorus that is removed in excess of what is found in the plants indicates that there may be some deposition of sludge at the bottom of the channels however no accumulation of sludge is observed. One explanantion may be that as the sludge degasses it floats to the top and is removed during harvesting operations.

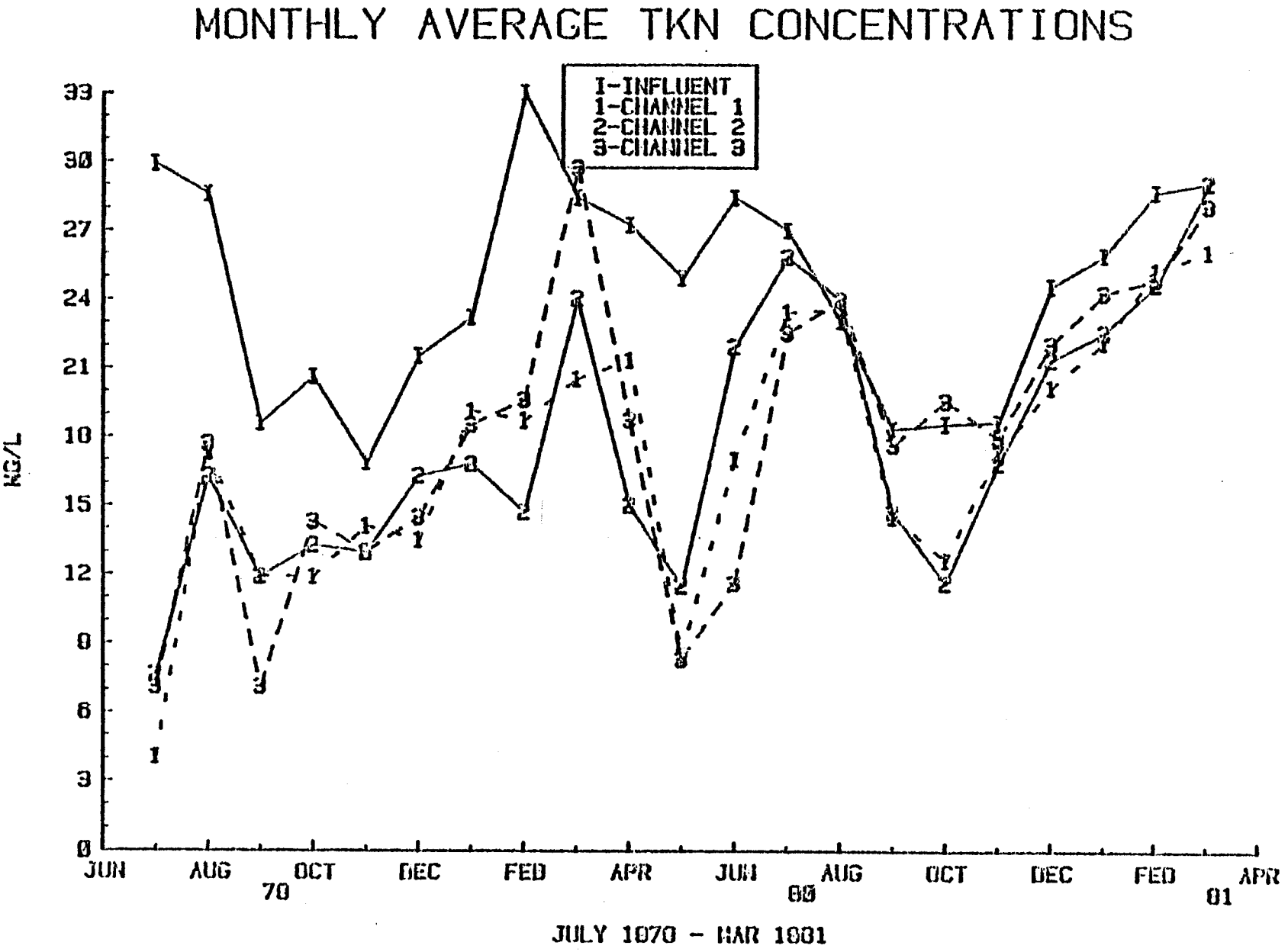


Figure 8 - Monthly average total kjeldahl nitrogen concentrations

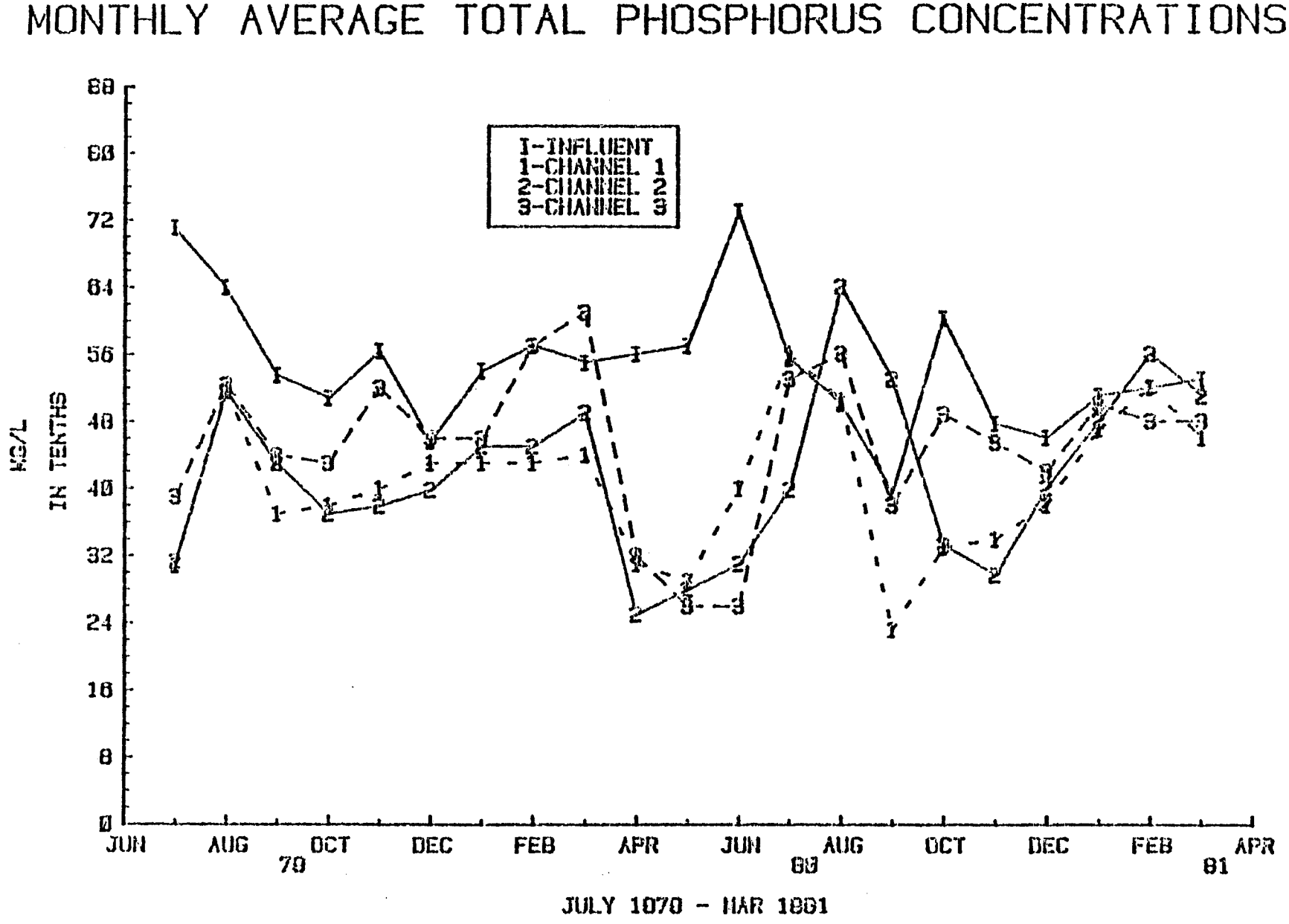


Figure 9 - Monthly average total phosphorus concentrations

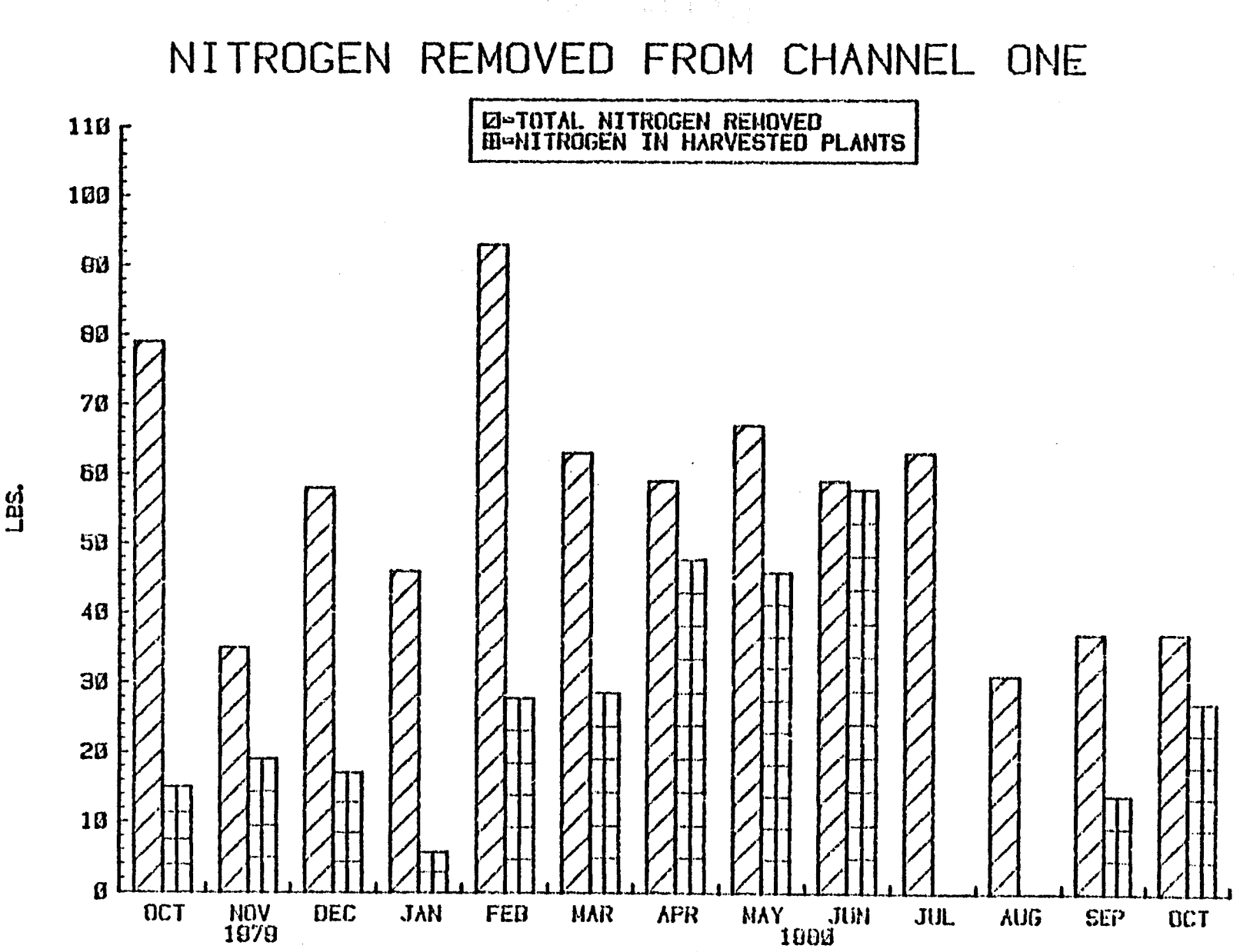


Figure 10 - This bar chart indicates the amount of total nitrogen removed from the wastewater and the amount of nitrogen found in the harvested plants from channel one. The bar representing the amount of nitrogen in the harvested plants assumes 4% nitrogen in the dry plant material. No harvest was accomplished in July and August.

TOTAL PHOSPHORUS REMOVED FROM CHANNEL ONE

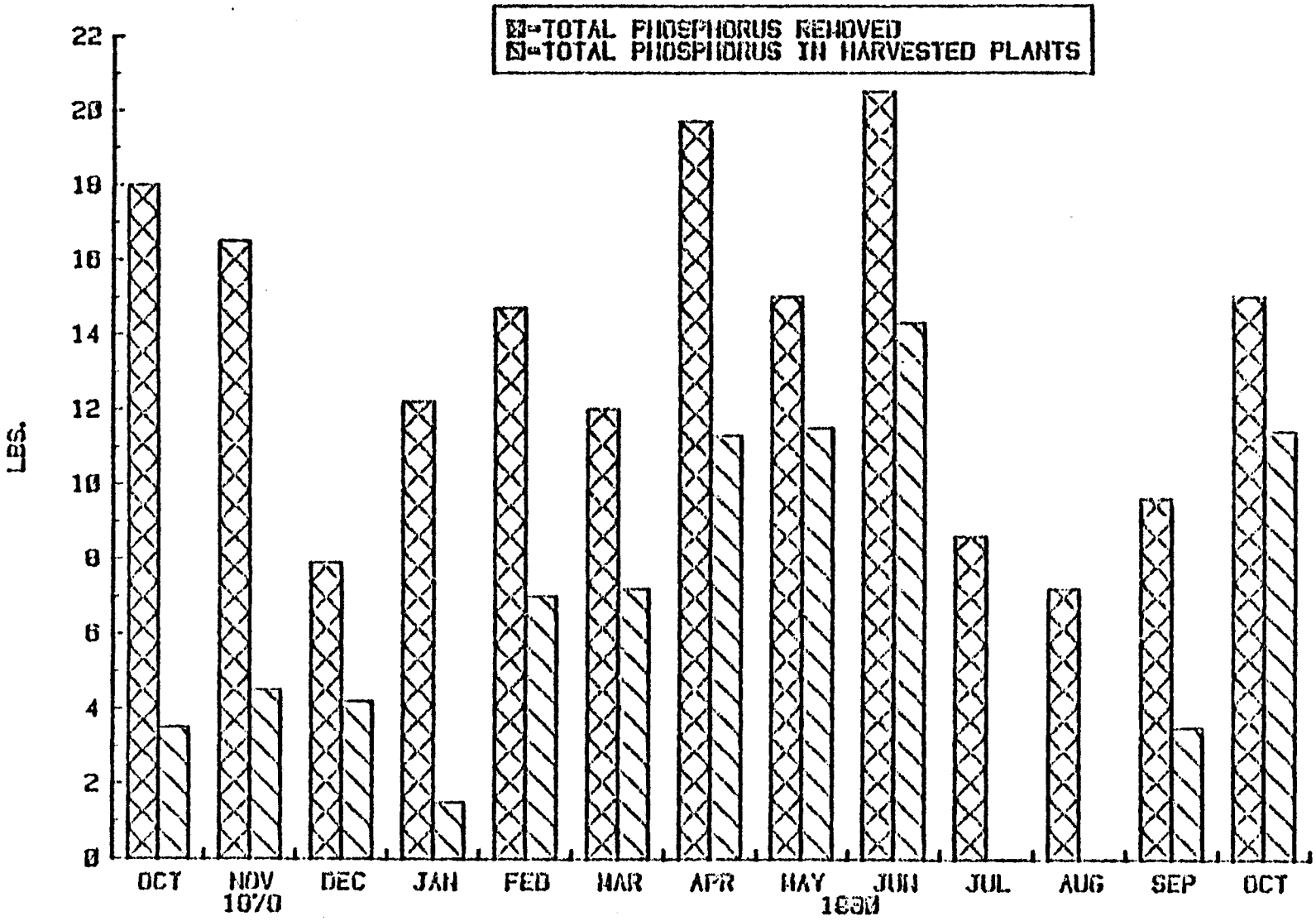


Figure 11 - This bar chart indicates the amount of total phosphorus removed from the wastewater and the amount of total phosphorus in the harvested plants from channel one. The bar representing the amount of total phosphorus assumes 1% phosphorus in the dry plant material. No harvest was accomplished in July and August.

In January 1981, the first set of trace metals were analyzed from the influent and effluent ends of each channel. Data was collected for the following trace metals: boron, calcium, magnesium, potassium, copper, iron, manganese, zinc, lead, cadmium, chromium and arsenic. See Table 3. The system demonstrated the greatest percent removal of lead (60-78% removal) while potassium showed the least percent reduction (1-4%). This system seems slightly more efficient than another study [7] using a continuous flow through system for removing boron while showing less efficiency at removing arsenic. Unfortunately due to the frost in January 1981, no tissue samples were taken, however tissue samples taken in the first quarter of 1980 indicated an abundance of iron in the water hyacinths. See Table 1. This abundance of iron appears to be normal for water hyacinths grown in wastewaters [8]. Disney water hyacinths also contain more zinc and less manganese than water hyacinths growing in natural stands [9], wastewaters [8], and nutrient solutions (see Table 1). Trace metal samples from the influent, effluent and tissues will continue on a once/quarter basis throughout the remainder of this study.

Productivity

From January 1980 - June 1980 Channels #1 and #2 were consistently harvested in the following manner: In Channel #1 the cells were sequentially harvested once a month while Channel #2 was harvested from the influent end only once a month. Channel #1 produced the equivalent of approximately 21.3 dry tons/acre-year (47.7 metric tons/hectare-year); Channel #2 produced 20.7 dry tons/acre-year (46.4 metric tons/hectare-year). Unfortunately the channels could not be harvested during the months of July and August, because of the moth infestation. Looking at the harvesting profile there does not seem to be any distinct advantage in one harvest pattern over the other in terms of productivity, one channel doing better one month, the other the next. See Fig. 12. The decline in growth in June, 1980, observed in Channel #2 is most likely explained by the moth infestation. Channel 3 which was restocked in May 1980 and not harvested until October 1980, produced only 16.20 wet tons during that period while Channel 1 produced 35 wet tons and Channel 2 produced 31 wet tons in that same six month time span. The difference in yield clearly indicates the importance of continued harvesting for maximum productivity.

TABLE 3

AVERAGE VALUES FOR JANUARY 1981

	Influent	#1	#2	#3	% Removal		
					#1	#2	#3
Boron mg/l	0.14	0.10	0.09	0.13	38.2	46.4	26.0
Calcium mg/l	33.6	27.1	34.3	35.5	30.5	14.7	15.8
Magnesium mg/l	5.6	6.1	6.1	6.1	6.2	39.4	13.4
Potassium mg/l	9.7	10.8	11.6	12.4	4.1	0	(2.0)
Copper ppb	27.9	27.9	20.8	31.4	13.8	36.4	11.0
Iron ppb	457.8	364.8	377.2	347.9	31.3	31.1	39.4
Manganese ppb	18.2	13.1	12.8	15.6	37.6	40.0	33.3
Zinc ppb	280.8	407.5	388.7	444.9	(24.7)	(15.4)	(26.2)
Lead ppb	12.8	4.9	6.7	3.5	66.7	60.0	78.0
Cadmium ppb	0.35	0.17	1.1	0.31	66.7	(133)	25.0
Chromium ppb	0.83	0.72	0.95	0.72	25.0	14.3	28.0
Arsenic ppb	0.88	0.88	0.88	0.88	12.5	14.3	26.7

Table 3 - Average trace metal concentrations sampled during January 1981

TOTAL HARVEST OCT 1979-OCT 1980

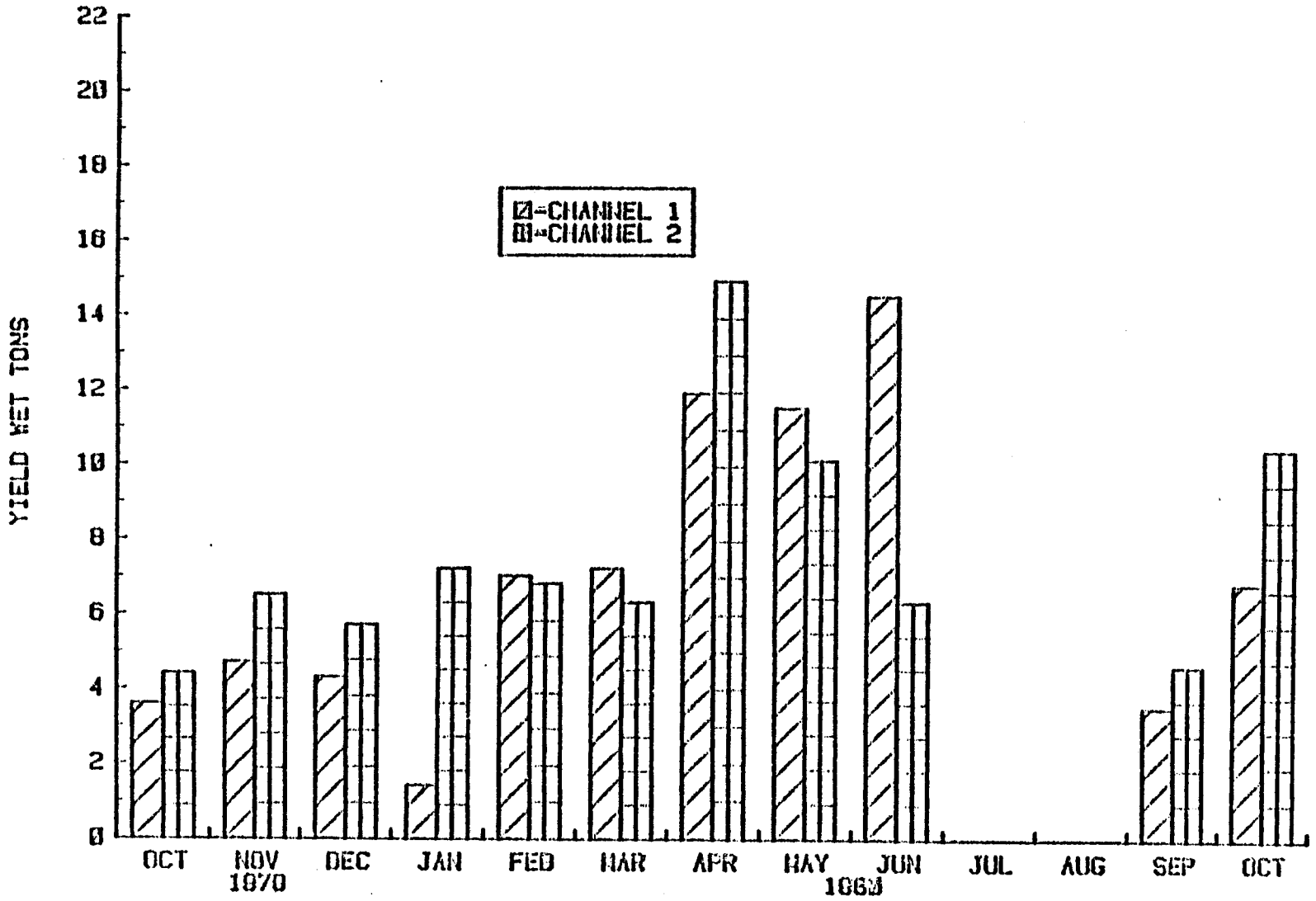


Figure 12 - This bar chart demonstrates the amount of harvest from channel one and channel two. Channel one was harvested from six points in the channel, while channel 2 was harvested from the influent end only.

Productivity and nutrient uptake are certainly related, however in a system such as this, with virtually unlimited nutrients for the plants, the growth rate of the hyacinths is mostly controlled by the environment particularly ambient temperatures. See figures 13, 14, 15. These graphs plot the monthly yield versus the average monthly ambient temperature, insolation, and water temperature. From these graphs the ambient temperature has the highest correlation coefficient with yield. A protective cover during the winter months should increase the yield of water hyacinths. In addition a cover provides the possibility of utilizing CO_2 enrichment, which according to studies at the University of Arizona can stimulate growth up to four times. [10]

Previous reports have indicated that water hyacinths can produce 40-60 dry tons/acre-year (89.6 - 134.4 dry metric tons/hectare-year) [2, 11, 12]. In addition to varying the harvest routines, and the restocking densities to optimize growth, the possibility that there may be something in Walt Disney World primary effluent that may be inhibiting or limiting growth is also under investigation. Future laboratory studies by University of Arizona under controlled conditions will indicate if there is growth inhibitor or a limiting element in the primary effluent.

Energy

Preliminary calculations indicate that the water hyacinth system would utilize less than 50% of the energy required for a conventional secondary treatment system utilizing activated sludge. See Table #4. These tables are average monthly energy requirements for the Water Hyacinth Systm and the Reedy Creek Utility Co. secondary treatment system scaled to one M.G.D. These are energy figures which do not consider the amount of energy produced by the conversion of water hyacinths to methane. Using 3.0 scf methane/lb. of volatile solids, [13] The water hyacinth system at 25 dry tons/acre-year (56.0 dry metric tons/hectare-year) would produce 1.4×10^8 BTU's/month per million gallons of wastewater treated each day. These preliminary calculations, based on biomass yields for one year, indicate that the water hyacinths could produce enough methane to operate the channels.

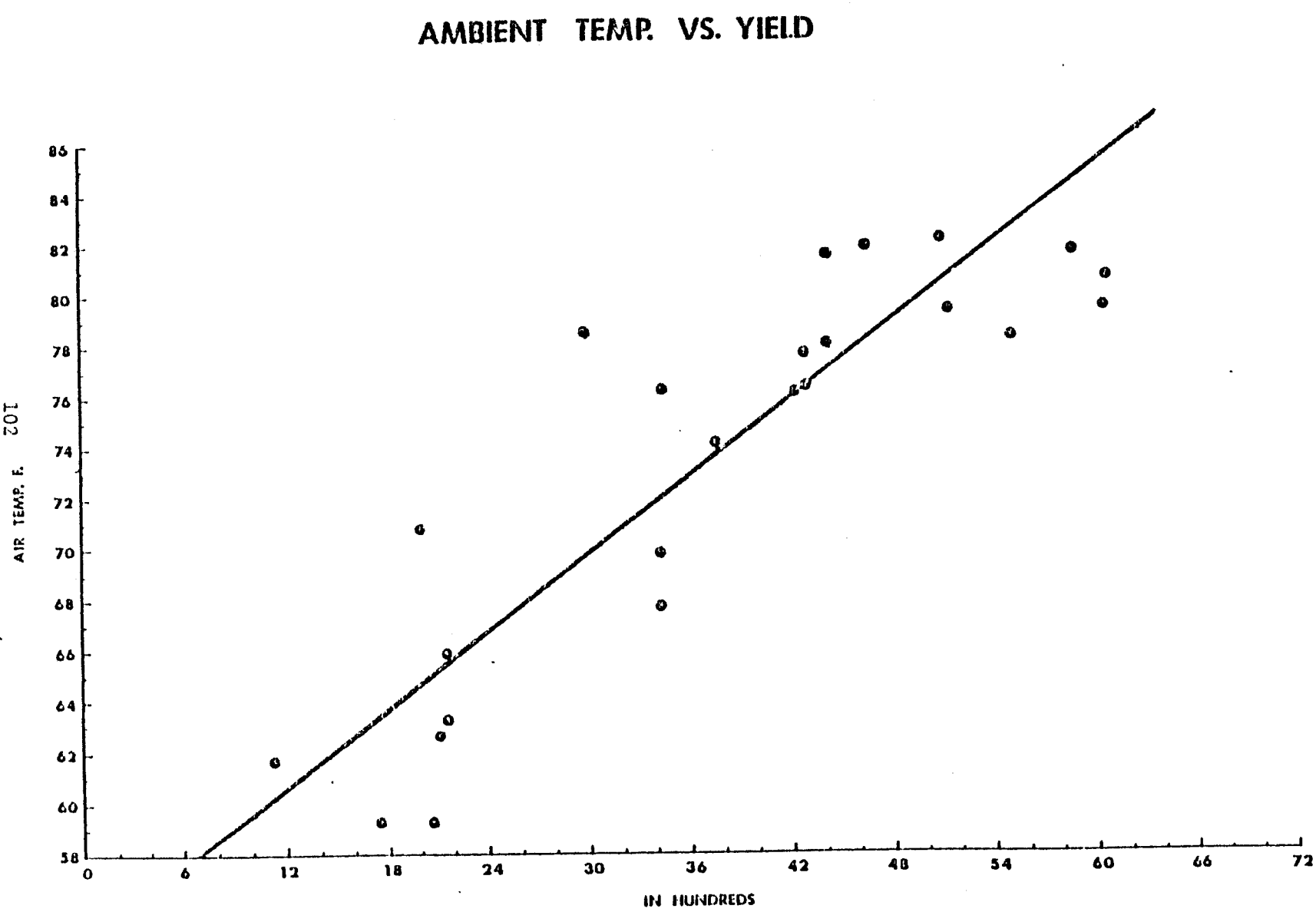


Figure 13 - Graph of the monthly yield versus the average ambient air temperature. The above least squares linear regression analysis has a correlation coefficient of 0.87.

INSOLATION VS. YIELD

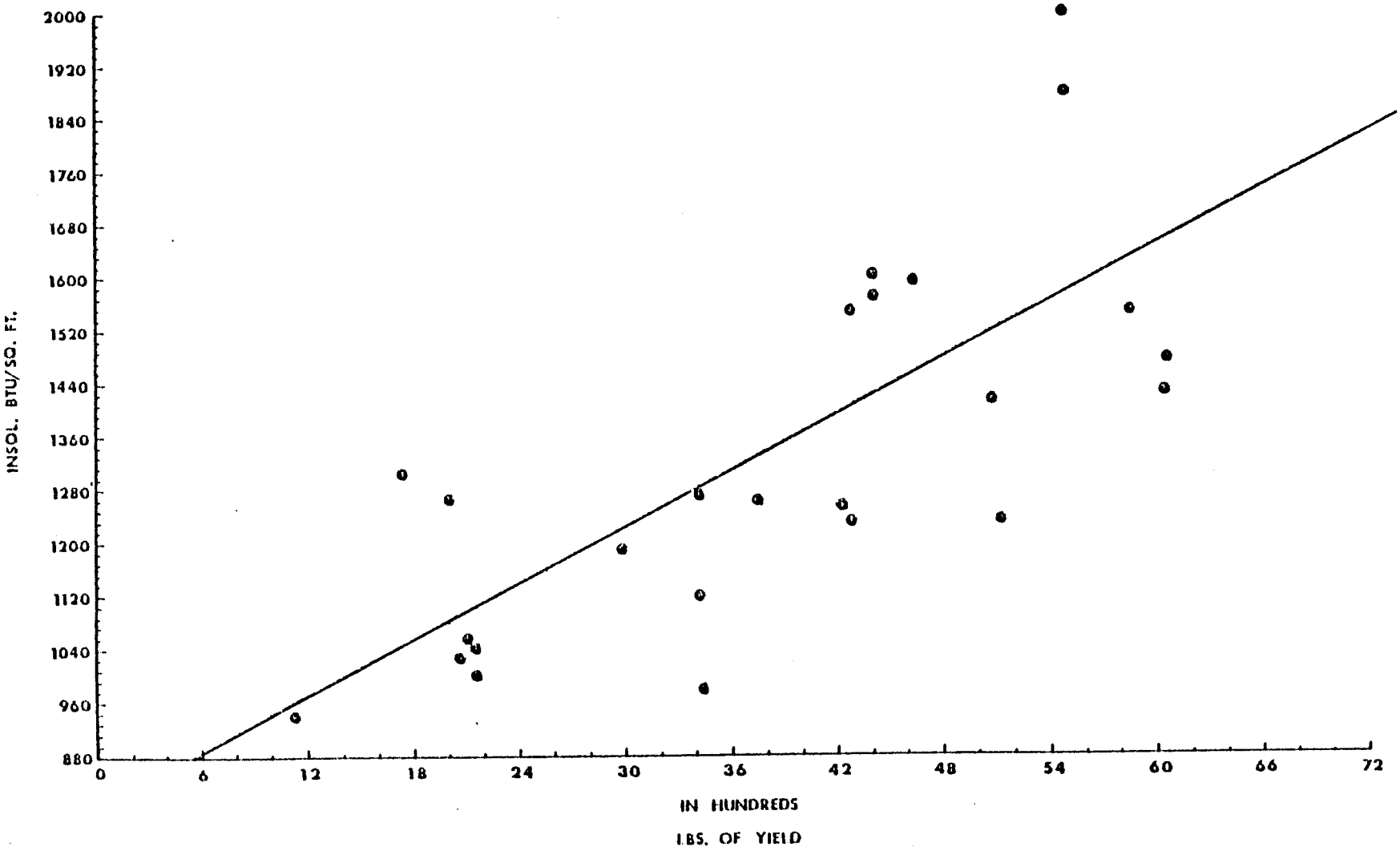
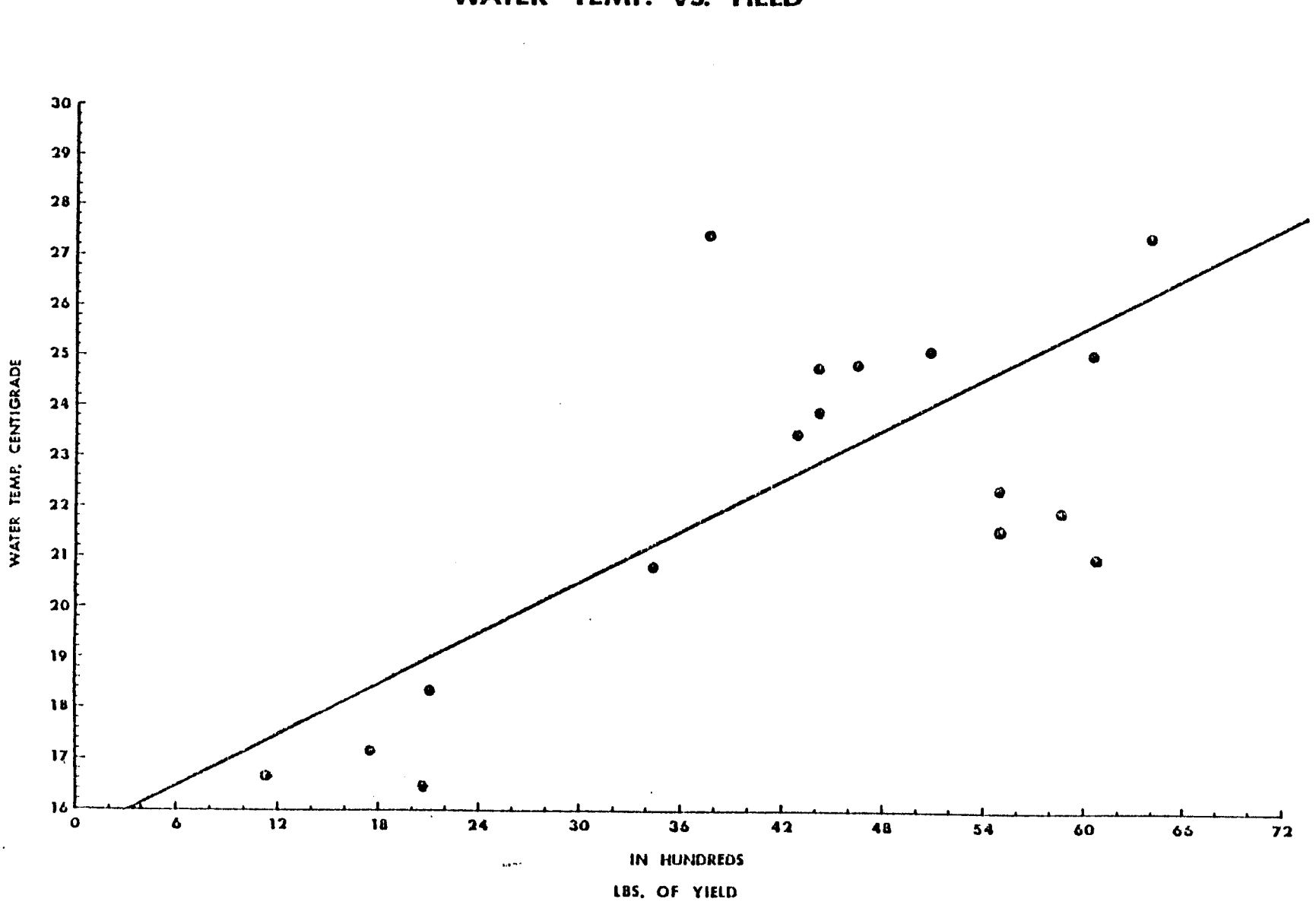


Figure 14 - Graph of the monthly yield versus average solar insolation. The above least squares linear regression analysis has a correlation coefficient of 8.78.

Figure 15 - Graph of the monthly yield versus the average water temperature. The above least squares linear regression analysis has a correlation coefficient of 0.78.



WATER HYACINTH WASTEWATER TREATMENT SYSTEM

TABLE 4

Energy requirements for the Water Hyacinth Wastewater Treatment System at 50,000 gpd for 3/4 Acre System (as a secondary treatment).

Influent Pump	1.8×10^6 BTUs/month
Effluent Pump	1.2×10^6 BTUs/month
Harvester	3.7×10^6 BTUs/month
Forage Truck	3.5×10^6 BTUs/month
	10.2×10^6 BTUs/month
AT 1 MGD using 15 acres (estimated)	1.30×10^8 BTUs/month

RCID Wastewater Treatment Plant energy requirements for secondary treatment at 3.3 MGD

Aeration Tanks	9.2×10^8 BTUs/month
Clarifiers	1.4×10^6 BTUs/month
	9.21×10^8 BTUs/month
AT 1 MGD (estimated)	2.7×10^8 BTUs/month

Table 4 - Energy requirements for the water hyacinth wastewater treatment system and the Reedy Creek Improvement District Wastewater Treatment Plant.

FUTURE OPERATIONS AND MILESTONE CHART

In addition to the 3 channel expansion of the water hyacinth wastewater treatment system, the future operations also plan for an advanced anaerobic dige stion process for converting most of the water hyacinths to methane gas. The Process Development Unit (PDU) is presently being designed by IGT under sponsorship of GRI. Installation and Operation of the PDU is scheduled for July 1982.

The revised milestone chart for the execution of the SERI contract is shown in Figure 16.

CONCLUSION

The Water Hyacinth System at Walt Disney World has demonstrated it does obtain secondary wastewater treatment standards. The first year's operation has produced good water quality and yielded the equivalent of 21 dry tons/acre-year (47.8 dry metric tons/hectare). The preliminary calculations based on one year's operation, indicate that the water hyacinth system not only utilizes 50% less energy than a comparable sized conventional secondary treatment system, but by converting water hyacinths to methane, the treatment system could be energy self-sufficient.

WATER HYACINTH PRODUCTION/SECONDARY & TERTIARY TREATMENT OF WASTE WATER TIME & MILESTONE CHART

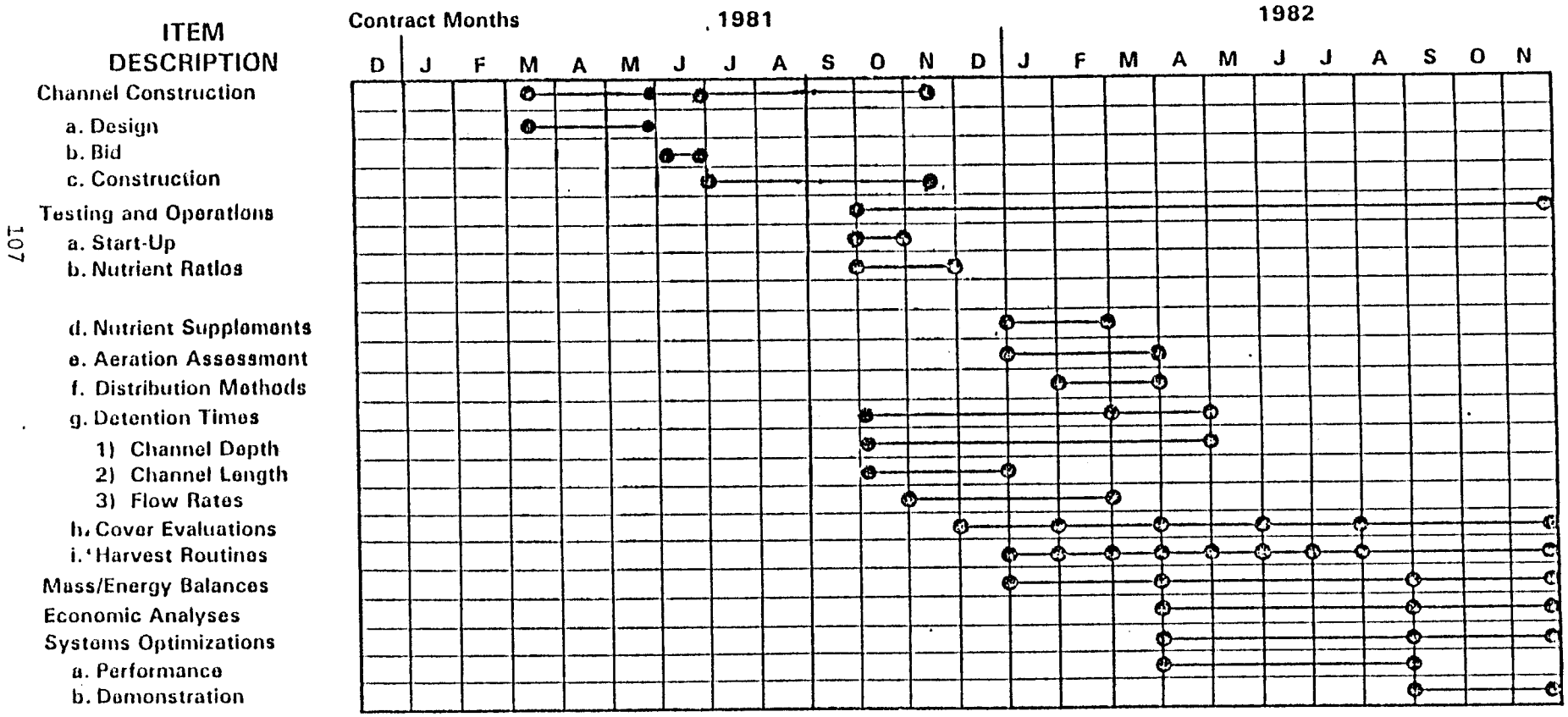


Figure 16

REFERENCES CITED

1. Finstein, M., "Microbial Ecosystem for Composting". Paper presented at 10th Annual Composting and Waste Recycling Conference, Compost Science/Land Utilization Conference, Los Angeles, Ca., May 5-7, 1980.

2. Ryther, J. H., "Cultivation of Macroscopic Marine Algae and Freshwater Aquatic Weeds," Progress Report prepared for the Solar Energy Research Institute Contract No. XR-9-8133-1, January 1980.

3. Phillips, J. M. and Hartung, R. F., "Solar Energy for Liquid and Gaseous Fuel Production by Means of Bioconversion," Final Report prepared for the Arizona Solar Energy Research Commission State Contract No. OEPAO - 368-77 (AG C2130), 1977.

4. Stewart, E. A., "Utilization of Water Hyacinths for Control of Nutrients in Domestic Wastewater Lakeland, Florida." Progress Report to Dawkins and Assoc., Orlando, Fla., Sept. 1979.

5. Swett, D. PhD., and Padera, C. Research Development Manager, Per Comm. Coral Ridge Properties, Division of Westinghouse, Coral Springs, Fla., Aug. 8, 1980.

6. Quimby, Jr., P. C. "Biological Control Technology Development," Proceedings, Research Planning Conference on the Aquatic Plant Control Program, New Orleans, LA. Miscellaneus Paper A-78-1, Office, Chief of Engineers, U.S. Army. Oct. 3-6, 1977.

7. Tridech, S., A. J. Englande, Jr., Michael Hebert, Jr., R. F. Wilkinson "Kinetics of Trace Contaminant Removal From Secondary Domestic Effluent by Vascular Aquatic Plant Systems" Paper presented at the Water Pollution Control Federation Conference, Las Vegas, Nevada, October 1, 1980.

8. Wolverton, B. C. and McDonald, R. C. "The Water Hyacinth: From Prolific Pest to Potential Provider" Ambio Vol. 8, No. 1 p. 2-9, 1979.

9. Boyd, Claude E. "Vascular Aquatic Plants for Mineral Nutrient Removal from Polluted Waters" Economic Botany 24: 95-103, 1970

10. Fontes, M. PH.D. Research Horticulturist, Per. Comm., Environmental Research Laboratory, University of Arizona, Tuscon, November 13, 1979.

11. Benemann, J.R., "Energy from Aquaculture Biomass Systems: Fresh and Brackish Water Aquatic Plants," Final Report to Office of Technology Assessment U.S. Congress, Task X, Oct. 10, 1979.

12. Wolverton, B. C., and McDonald, R. C., "Water Hyacinth (Eichhornia crassipes) Productivity and Harvesting Studies" Section I Part 3 ERL Report No. 171 TMX-72729, NASA, NSTL Bay St. Louis, Mo. Mar 1967.

13. Chynoweth, D. P. and Ghosh, S., "Energy Conversion of Sewage Sludge and Water Hyacinth" Report presented at Program Review Meeting of the Water Hyacinth Wastewater Treatment System Walt Disney World, Fla. Nov. 11, 1980.

CULTIVATION OF MACROSCOPIC MARINE ALGAE

J. H. Ryther
Woods Hole Oceanographic Institution
Woods Hole, Massachusetts 02543

ABSTRACT

High yields of Gracilaria were obtained in cultures suspended by aeration with rapid exchange of enriched seawater. Attempts were made to reduce the high cost inputs of aeration, seawater flow and nutrients and still maintain high yields. Physiological research was required to understand the function of those inputs and how to reduce them. Gracilaria soaked in nutrient solutions for several hours was capable of storing sufficient nutrients for subsequent growth for one-two weeks. Intermittant aeration for a few hours/day was equally effective as continuous aeration for growth enhancement. Rapid seawater exchange is necessary to maintain pH control, since Gracilaria has little ability to utilize bicarbonate and virtually no free CO_2 exists at pH 9.0, commonly reached in high-yield, low seawater exchange cultures. Ulva and other species that use bicarbonate readily may be better marine biomass candidates than those like Gracilaria and Macrocystis that lack that ability.

INTRODUCTION

The highest yields of marine biomass that have yet been reported to date on a sustained basis are those of the red seaweed Gracilaria tikvahiae. Grown in suspended culture by vigorous aeration and with 25-volume exchanges per day (retention time 0.04 days) of enriched seawater, the seaweed produced the equivalent of 129 dry tons per hectare per year, grown throughout the year in 50 1 outdoor cultures in Fort Pierce, Florida [1]. Since that time, attempts have been made to reduce high cost and high energy inputs to the culture system, such as continuously pumped seawater nutrients and air, while still achieving high yields. This approach has required a program of basic research on the physiology and nutrition of Gracilaria, and some other species of seaweed, in order to determine exactly what the requirements are for optimal growth of the alga, how those requirements are met by the emperically-derived methods used to obtain the high yields, and how those requirements might be met by other, less costly procedures.

Nutrient Uptake Kinetics

Nutrient-starved Gracilaria, placed in an enriched seawater solution in either the light or dark, are able to soak up sufficient ammonium-nitrogen in 6-8 hours to double its internal nitrogen content (i.e., from ca. 1.5 to 3.0% of dry weight) and permit its subsequent growth at non-nutrient-limiting rates for one to two weeks (depending upon the growth rate as

determined by light, temperature and other factors) in unenriched, flowing seawater. NO_3^--N is taken up less efficiently than is NH_4^+-N (Figure 1) [2].

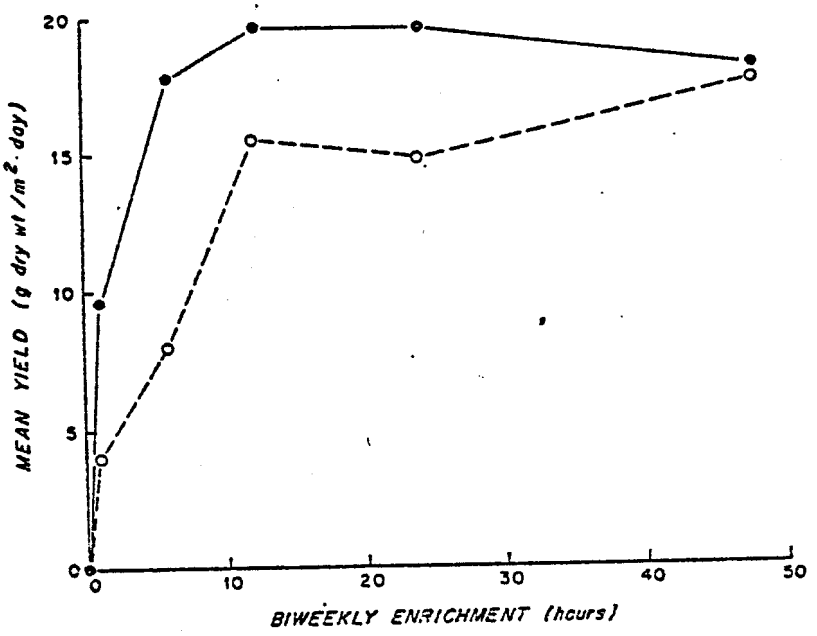


Figure 1. MEAN YIELD OF NITROGEN-STARVED GRACILARIA IMMERSED IN NUTRIENT MEDIUM CONTAINING AMMONIUM-NITROGEN (CLOSED CIRCLES) AND NITRATE-NITROGEN (OPEN CIRCLES) EVERY TWO WEEKS FOR THE PERIODS OF TIME INDICATED.

Seaweed taken from a flowing seawater culture may thus be removed periodically, soaked for a few hours in a static nutrient solution, and then returned to the culture system, the process coinciding with the periodic harvesting back of the culture. This would not only conserve nutrients, those unused remaining in the nutrient bath rather than being lost in the flowing seawater effluent, but it would also prevent or retard the growth of undesirable epiphytes brought into the system as spores in the seawater on the cultured seaweeds and the culture system, a chronic problem in seaweed culture

Initially, the nutrient solution consisted of nitrogen, phosphorus, chelated iron and a mixture of trace metals. Subsequently it was found that the same growth was obtained with NH_4^+-N alone as with the complete enrichment medium (Table 1), the unenriched seawater apparently containing an adequate supply of the other nutrients. Enrichment with nitrogen alone would, of course, represent a considerable cost savings, but whether or not this could be done in other, less nutrient-rich sources of seawater remains to be seen. However, the liquid residues left from anaerobic digestion of Gracilaria for methane production appear to contain all the nutrients necessary for the growth of the seaweed and their recycling has been advocated as a low-cost nutrient source for biomass production [3].

Table 1. EFFECT OF EXPOSING GRACILARIA 24 HOURS PER WEEK TO THREE DIFFERENT NUTRIENT MEDIA ON SUBSEQUENT GROWTH (MEAN YIELD: g dry wt/m^2.day).

Dates (1981)	Media N	N + P	N + P + Fe + trace elements
4/24 - 5/1	12.7	12.9	11.9
5/1 - 5/8	13.0	14.7	13.0
5/8 - 5/18	21.0	19.3	16.7
5/18 - 5/25	17.0	19.0	16.6
Mean	15.9	16.5	14.6

Effect of Aeration/Mixing

Gracilaria is maintained in suspended culture by aeration along the long axis of the culture tank bottom. What purpose this serves is not known, but apparently does not derive from the air itself, since the same growth enhancement is obtained from seaweed kept in suspension by the action of a paddle wheel [4]. It may expose a larger density of seaweed to sunlight than would be possible in an unmixed culture, or it may increase the exposure of the plants to CO_2 and/or other nutrients. Whatever the function, aeration is a major cost and energy input that should be reduced to a minimum level consistent with high yield. Table 2 shows that intermittent aeration, for as little as six hours per day, under two different periodicities, results in the same yields of Gracilaria as does continuous aeration, but that yields decreased in cultures aerated for only 5 minutes per hour for a total of two hours per day. The effect of aeration periods intermediate between two and six hours per day and during daylight hours only will next be investigated.

Table 2. EFFECT OF AERATION ON YIELD OF GRACILARIA GROWN AT TWO RETENTION TIMES OF ENRICHED FLOWING SEAWATER (MEAN YIELDS 4/9-5/4/81 in g dry wt/m^2.day).

Aeration (hours/day)	Retention(days) 0.1	1.0
0	18.6	8.5
6 (5 min/hr)	23.0	9.9
6 (15 min	32.9	12.5
6 (2 hrs, 3 x/day, daylight	34.0	14.2
12 (daylight)	36.5	14.7
24	37.3	14.7

Whatever the effect, it is clear from Table 2 that some degree of aeration or the mixing it produces is necessary for high sustained yields of the seaweed. Beginning in May, 1981, with the completion of eight new 5 m^2 concrete ponds, Gracilaria has been cultured passively on the pond bottom with no mixing or agitation of the seawater, as is done commercially in southern Taiwan [5]. Yields under different growing conditions (seaweed density, water depth, method of nutrient supply and mixing of the seawater) has resulted to date in some yield differences (Table 3), but in all cases they have been significantly lower than those obtained in suspended cultures at the same retention time (see Table 2)

Table 3. YIELDS FROM POND-BOTTOM CULTURE OF GRACILARIA IN 5 m^2 CONCRETE TANKS WITH SEAWATER RETENTION TIME OF ONE DAY.

Water depth (cm)	Density (kg wet wt/m^2)	Nutrient supply	Aeration[1]	Mean yield (g dry wt/m^2.day) 5/4 - 6/1/81
77	3	pulsed	yes	11.9
51	3	"	yes	9.1
26	3	"	yes	4.5
77	1.5	"	yes	10.3
77	4.5	"	yes	8.7
77	6	"	yes	4.9
77	3	"	no	8.2
77	3	continuous	yes	8.9

[1]Gentle aeration to mix water but not disturb algae.

Seawater Retention, pH and Carbon Dioxide

The one factor that has been found to date the most important in affecting the growth and yield of Gracilaria is seawater exchange rate (retention time). Again the reason is not obvious, but does not appear to be related to the nutrient supply, since the effect is observed when nutrients are added at a constant rate separately from the seawater supply, or when nutrients are supplied by soaking the seaweed periodically for a few hours in a static, enriched seawater reservoir, as discussed above. Figure 2 is a summary of experiments showing the relationship between yield and retention time, including cultures ranging in size from 50 to 600,000 liters and with both continuously-enriched and pulse-fed cultures.

From those data and an assumed pumping cost of $2.60/thousand cubic meters ($10/million gallons), it may be estimated that for the methane generated from Gracilaria cultures one meter deep, the cost of seawater pumping alone would range from $9354/thousand cubic meters ($265/thousand scf) of 100%

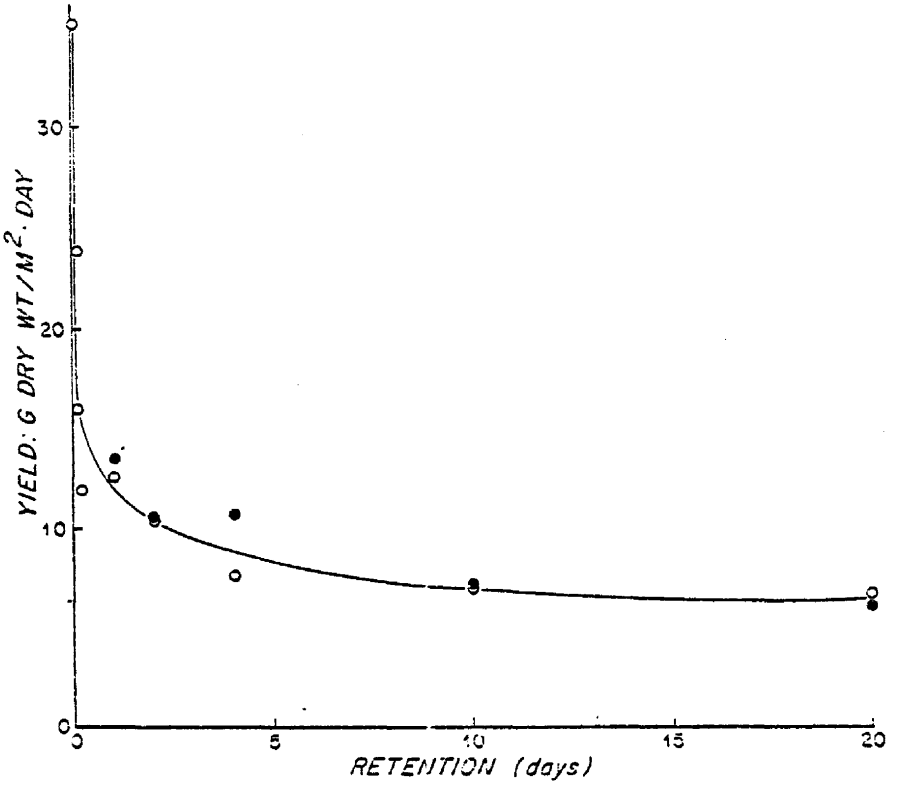


Figure 2. YIELDS OF GRACILARIA AS A FUNCTION OF SEAWATER RETENTION TIME. OPEN CIRCLES = CONTINUOUS ENRICHMENT: CLOSED CIRCLES = PULSED NUTRIENTS FOR 24 HOURS EVERY TWO WEEKS.

methane at a retention time of 0.04 days to about $100/thousand cubic meters ($2.80/thousand scf) of methane at a 20-day retention time (assuming 0.2 l 100% methane/g volatile solids). However the area required for the seaweed farm would range from 40 hectares/10^6 m^3 of methane produced (2.8 acres/10^6 scf) at the 0.04 retention time, to nearly 200 hectares/10^6 m^3 methane (14.1 acres/10^6 scf) at the 20-day retention time. Clearly, it would be desirable to achieve the high yields possible at very rapid exchange rates with much less water flow. First, however, it is necessary to understand the relationship between yield and water exchange

The only essential nutrient not provided in the artificial enrichment normally used to culture Gracilaria and other seaweeds is carbon dioxide. Seawater of normal salinity (30-35‰) contains about 2 m moles/liter of total CO_2 which exists in the equilibrium:

$$CO_2(\text{diss.}) \leftrightharpoons H_2CO_3 \rightleftharpoons HCO_3^- \leftrightharpoons CO_3^= \qquad (1)$$

Seawater one-meter deep thus contains 24 g C/m^2 which could theoretically support the growth of 48 g ash-free dry weight (50% carbon) or about 74 g

total dry weight of Gracilaria, a potential yield of 74 g dry wt/m^2.day with a retention time of one day. However, removal of free CO_2 during the photosynthetic growth of algae increases the pH; the slower the circulation of seawater through the seaweed the higher the pH rises (Table 4). At pH > 9.0, there is almost no free CO_2 in seawater [6] and its rate of dehydration from bicarbonate to maintain the equilibrium is so slow that it becomes limiting to photosynthesis and growth [7].

Table 4. MEAN, DAYTIME pH IN GRACILARIA CULTURES AT DIFFERENT RETENTION TIMES.

Time	Retention (days) 0.1	1.0
0800	8.2	8.1
1030	8.4	8.6
1315	8.7	9.0
1435	8.7	9.1
1530	8.8	9.1
1615	8.7	9.1

Some seaweeds are able to utilize bicarbonate directly in photosynthesis. Measurement of photosynthesis (ΔO_2 by oxygen probe in a closed, recirculating seawater chamber) at four pH levels maintained with TRIS buffer indicates, however, that Gracilaria can use little or no bicarbonate, with photosynthesis at pH 9.0 only 19% of that at pH 7.5 (Table 5).

Table 5. EFFECT OF pH ON PHOTOSYNTHESIS OF GRACILARIA AND ULVA, MEASURED BY OXYGEN INCREASE AFTER ONE HOUR. FIGURES ARE RELATIVE TO INCREASE AT pH 7.5.

pH	Gracilaria	Ulva
7.5 - 7.6	100	100
8.0 - 8.2	74	104
8.6	46	92
9.0 - 9.1	19	92

This, then, would account for the poor growth of Gracilaria at low seawater exchange rates and at the accompanying high pH levels.

Bicarbonate uptake and its internal dehydration to CO_2 requires the enzyme carbonic anhydrase. Osterlind [8] has shown that the enzyme is not always present but must be induced in certain unicellular algae before they can utilize bicarbonate. The question remains of whether carbonic anhydrase

can be induced in Gracilaria and/or other species of seaweeds, whether there are species or clones of Gracilaria that naturally contain the enzyme, or whether strains of the alga capable of using bacarbonate can be naturally selected for or genetically produced. In that connection, it would be interesting to see whether fast-growing strains of G. tikvahiae selected by Van der Meer in Halifax [9] may be fast growing because of that ability.

Other species of seaweed do utilize bicarbonate readily and continue photosynthesis and growth at high pH. The green alga Ulva lactuca, was found to produce oxygen at pH 9.1 at 72% the rate at pH 7.5 (vs. 19% for Gracilaria) (Table 5). Ulva would be an ideal candidate for marine biomass production for that reason and because of its high inherent growth rate and potential yield, were it not for the fact that the alga normally becomes reproductive and sheds a large fraction of its biomass as microscopic gametes or zoospores as often as once a week when it is growing rapidly. Dr. Howard Levine (U. Mass.) has kindly provided the author with a sample of Ulva lactuca from a population which he has never observed to become reproductive or to bear fruiting bodies. In the several weeks it has since been grown in our culture system, it has also remained sterile, in contrast to several other strains of the same species grown under the same conditions. A permanently sterile clone of Ulva, as may now be available, could represent an important contribution to the marine biomass field.

The above observations on bicarbonate utilization by Gracilaria and Ulva are not original to the present author. Almost exactly the same results with both species were reported in 1963 by L. R. Blinks [10], who also described similar experiments with 22 other species of seaweeds. A complete spectrum was found in the ability of the different species to utilize bicarbonate, with Ulva and Gracilaria, among others, representing the two extremes. The giant kelp, Macrocystis, another popular candidate for marine biomass production, photosynthesizes only marginally better than Gracilaria at pH 9.0. This basic characteristic of seaweeds may thus be an important criterion for the selection of candiadate species for marine biomass production, if the systems used for such purposes are to consist of extensive marine farms of high growth rate, high yield algal crops maintained in seawater that, for logistic or economic reasons, cannot be rapidly exchanged.

REFERENCES

1. Lapointe, B. E. and Ryther, J. H.: Some aspects of the growth and yield of Gracilaria tikvahiae in culture. Aquaculture, 15, 185-193 (1978).

2. Ryther, J. H., Corwin, N., DeBusk, R. A. and Williams, L. D.: Nitrogen uptake and storage by the red alga Gracilaria tikvahiae. Aquaculture, (in press).

3. Hanisak, M. D.: Recycling the residues from anaerobic digesters as a nutrient source for seaweed growth. Bot. Mar., (in press).

4. Neish, A. C., Shacklock, P. F., Fox, C. H. and Simpson, F. J.: The cultivation of Chondrus crispus. Factors affecting growth under greenhouse conditions. Can. J. Bot., 55, 2263-2271 (1977).

5. Shang, Y. C.: Economic aspects of Gracilaria culture in Taiwan. Aquaculture, 8, 1-7 (1976).

6. Harvey, H. W.: The chemistry and fertility of sea waters. The University Press, Cambridge, 1960, 240 pp.

7. Birmingham, B. C. and Coleman, B.: Measurement of carbon dioxide compensation points of freshwater algae. Plant. Physiol., 64, 892-895 (1979).

8. Osterlind, S.: Inorganic carbon sources of green algae. VI. Further experiments concerning photoactivation of bicarbonate assimilation. Physiol. Plant., 5, 403-408 (1952).

9. Patwary, M. and Van der Meer, J.: Improved biomass yields of Gracilaria tikvahiae through genetic modification of thallus morphology. Paper presented at 20th Northeast Algal Symposium, MBL, Woods Hole, MA April 11-12, 1981.

10. Blinks, L. R.: The effect of pH upon the photosynthesis of littoral marine algae. Phycologia, 57, 126-136 (1963).

WETLAND BIOMASS PRODUCTION

D. C. Pratt
University of Minnesota Botany Department
1445 Gortner Ave.
St. Paul, MN 55108

ABSTRACT

The use of wetlands to produce biomass crops has been the focus of several research programs at the University of Minnesota. There are over 6 million hectares of peatlands in the northern lakes states, Minnesota, Michigan and Wisconsin. Currently only 2.7% of Minnesota peatlands are utilized for crop production. Typha spp. (cattails), Phragmites communis (reed grass), Scirpus spp. (rushes), Carex spp. (sedges), and Phalaris arundinacea (reed canarygrass), Alnus spp. (alder) and Salix spp. (willow) are some species being considered as possible wetland energy crops. Using wetlands, including peat and wet mineral soils, for the production of energy crops has several advantages:

1) Substantial wetland acreage is available,
2) Wetland crops will not be in competition with traditional crops for prime agricultural lands,
3) Wetlands are naturally very productive habitats, often functioning as nutrient sinks,
4) Wetland crops represent a less destructive alternative than peat mining,
5) A mixture of native wetland plants can be used, avoiding the traditional monoculture approach.

Typha looks particularly promising; it is easily propagated from seed or rhizomes, and is very productive. The total biomass of natural Typha stands often exceeds 40 dry tons/hectare; yields from stands established from rhizome pieces range from 25-30 tons/hectare the first year while stands established from seed yield 8-12 t/ha the first season and 15-20 t/ha by the second season.

The current focus for Typha is the development of optimal land preparation, planting and management techniques. Alternatives are first tested in small replicated field plots; the most successful techniques will then be used to establish larger stands.

Studies of harvesting methods, biochemical conversion, land use considerations, possible environmental constraints, and the overall economics of wetland energy crop production are currently under way, administered by the University's Bio-Energy Coordinating Office (BECO). The major long term objective of the cooperative research program is to develop an efficient and renewable energy system using available resources while minimizing damage to the environment.

BACKGROUND

Productivity of Wetland Plants

Table 1. STANDING CROP IN NATURAL STANDS OF POTENTIAL WETLAND ENERGY CROPS.

Species	Dry Weight, gm^{-2}			Location	Reference
	Above Ground	Below Ground	Total		
CATTAILS					
Typha x glauca	1440	2650	4090	Minnesota	6
	2320	2400	4720	Minnesota	4
	1361	-	-	New York	7
	2106	-	-	Iowa	2
T. latifolia	428-2252	-	-	Southeast, U.S.	8
	1400	503	1903	Wisconsin	3
	500-2000	200-1400	800-3400	Czechoslovakia	9
T. elephantina	975-2464	1542-5269	-	India	10
T. angustifolia	564-1647	306-2861	4508	USSR	11
	1118	-	-	England	12
BULRUSH					
Scirpus fluviatilis	852	429	1281	Wisconsin	13
	450	429	1833	Iowa	14
	466	-	-	Iowa	2
REED					
Phragmites communis	1110	-	-	Iowa	14
	1118	-	-	Minnesota	15
	1115	-	-	Czechoslovakia	9
REED CANARYGRASS					
Phalaris arundinacea	1370	-	-	New York	16
	870	-	-	England	17
	1353	-	-	Wisconsin	13
SEDGE					
Carex atherodes	1160	-	-	Minnesota	18
C. lacustris	738	-	-	Minnesota	18
	940	134	1074	Wisconsin	13
	857	161	1018	New York	19
	1145	-	-	New York	20

Natural Stands

Wetlands dominated by Typha and other emergent macrophytes are one of the most productive natural systems in the temperate zone (1). Above ground standing crops in natural stands often exceed 15 dry tons/hectare (2, 3) while total biomass in the most productive stands of over 40 dry tons/hectare have been reported (4, 5). Table 1 summarizes productivity estmates of potential wetland crop including cattails, bulrushes, reeds, reed canary grass, and sedges.

Typha is Particularly Attractive as a Biomass Crop. Table 2 presents a summary of Typha yields from different regions. Reports of above ground dry weight range from 428 g/m^2 to 2464 g/m^2. These data should be interpreted cautiously, however, because samples were collected at different times during the season, in different years, using various sampling techniques. In some cases sample size was not sufficiently large to insure that results are representative of yields over an entire region.

Table 2. CATTAIL YIELDS IN MANAGED STANDS

Species	Dry Weight, gm^{-2} Above Ground	Below Ground	Total	Description
T. x glauca	1486	5455	6941	Rhizomes, $1.5m^2$ paddies soil mix + fertilizer, Minnesota (5)
T. x glauca	810-1540	2670-3200	3670-4210	Rhizomes, $1.5m^2$ paddies, soil mix + fertilizer, MN (21)
T. x glauca	1298-1469	1346-1762	2644-3231	Rhizomes, $1.5m^2$ paddies, peat + fertilizer, MN (22)
T. latifolia	2673-3349	1973-2581	5254-5802	Rhizomes, outdoor hydroponic cultures, Czechoslovakia (23)
T. latifolia	920-1200	-	-	Transplanted seedlings, second year, Florida (24)
T. x glauca	1268-1448	519-840	1697-2268	Seed, $1.5m^2$ paddies, second year, MN (22)

Typha Yields in Managed Stands

Table 2 presents a summary from the literature of Typha productivity in managed stands established with rhizomes, seedlings and seed. Generally total biomass equals or exceeds figures from natural stands. Obviously factors such as method of stand establishment (rhizomes, seedlings, seed), nutrient availability, planting density and management techniques have a tremendous effect on final yields and the yields obtained from small, carefully managed stands cannot necessarily be achieved in field trials. A field study was initiated in 1980 to examine some of these variables.

Peatland Stand Establishment Study. Typha was established in a northern Minnesota peatland using rhizomes and seed. The influence of planting density, substrate preparation, and fertilizer application on the seasonal development and productivity of trial plots was investigated (25). Table 3 summarizes some of the results of this study. Substrate preparation (rotovated or excavated), fertilizer application and increased planting density resulted in significantly increased yields. A maximum yield of 16 tons/hectare was obtained with a late May planting date using a planting rate of 25 rhizomes/m^2 with 90, 158 and 300 kg/ha of N, P, and K respectively. Although final yield increased with planting density stand establishment using relatively low planting rates may be cost effective.

PROJECT OBJECTIVES

A multidisciplinary team of researchers from the Departments of Botany, Ecology and Horticulture at the University of Minnesota is working on wetland crop production. The major objectives are to develop land preparation, propagation, planting and management practices that optimize sustained yield while minimizing energy costs and environmental degradation. While the initial focus is on the continued development of cattails as a commercial crop, several other potential wetland biomass crops will be considered including reeds, bulrushes, and sedges.

OUTLINE OF RESEARCH PROGRAM

I. Selection and Propagation of Wetland Biomass Crops
   - A. Identification of Promising Species
   - B. Selection of Highly Productive Individuals
   - C. Micropropagation Studies
   - D. Macropropagation Studies
   - E. Production of Commercial Scale Quantities

II. Field Trials
   - A. Site Preparation Methods
   - B. Planting Techniques
   - C. Fertilizer Requirements
   - D. Insect and Weed Control Techniques
   - E. Harvesting Schedule and Regrowth

III. Establishment of Large Plots (Year 2)
   - A. Site Selection
   - B. Site Preparation and Planting
   - C. Monitor Growth
   - D. Monitor Environmental Effects

TABLE 3 EFFECTS OF TREATMENTS ON STANDING CROP AND DENSITY[a]

Area tested	Density vs density	Standing crop	Final density
Effect of Planting Density			
T. latifolia Rotovated,fertilized	$9/m^2$ vs $25/m^2$	s	s
	$4/m^2$ vs $25/m^2$	s	s
	$1/m^2$ vs $25/m^2$	s	s
	$4/m^2$ vs $9/m^2$	s	ns
	$1/m^2$ vs $9/m^2$	s	s
	$1/m^2$ vs $4/m^2$	ns	ns
T. x glauca Rotovated,fertilized	$1/m^2$ vs $4/m^2$[b]	ns	ns
T. latifolia Excavated,fertilized	$4/m^2$ vs $9/m^2$	ns	ns
	$1/m^2$ vs $9/m^2$	s	s
	$1/m^2$ vs $4/m^2$	s	ns
Effect of Substrate Preparation			
Rotovated vs excavated			
T. latifolia, fertilized	$9/m^2$	ns	ns
T. latifolia, fertilized	$4/m^2$[b]	ns	ns
T. latifolia, fertilized	$1/m^2$[b]	ns	ns
Rotovated vs unrotovated			
T. latifolia, fertilized	$9/m^2$	s	ns
T. latifolia, unfertilized	$9/m^2$	s	ns
T. x glauca, fertilized	$1/m^2$[b]	s	s
Effect of Fertilizer			
Fertilized vs nonfertilized			
T. latifolia	$9/m^2$	s	ns
T. latifolia	$9/m^2$	s	ns
Effect of Species Type			
T. latifolia vs *T. x glauca*			
Rotovated, fertilized	$4/m^2$[b]	ns	ns
Rotovated, fertilized	$1/m^2$[b]	ns	ns

[a]s = significant difference, ns = no significant difference, = 0.05.
[b]T-test.

CURRENT FOCUS

Selection

Identification of Promising Genotypes

Cattail rhizomes were collected from productive natural stands identified from previous studies (26). The rhizomes were planted in peat-filled paddies using a 6 x 6 latin square design in order to compare productivity under identical growing conditions. This experiment will also provide us with sufficient planting material for more extensive experiments in subsequent seasons.

Propagation

The three stages in the propagation work include:
1. Establishment of Typha explants in tissue culture with formation of callus tissue.
2. Proliferation of individual plantlets in tissue culture, and
3. Establishment of these plantlets in soil and then in the field.
Current work with Typha is now at step 1.

Attempts to micropropagate Typha began in December, 1980. Four trials have been conducted, averaging 160 explants each. Stock plants have also been grown from seed and rhizomes, some under sterile conditions, to serve as explant sources. There are a large number of factors which must be considered in starting micropropagation work with a new species. Some of these factors and some of the possible alternatives which are being used with Typha include: 1) Part of plant being used as the explant, 2) Culture medium, 3) Plant growth regulators, 4. Environmental conditions, 5) State of medium, and 6) Decontamination procedures.

Establishment of Typha rhizome explants in a sterile tissue culture environment has been slow. Explants survive longest when kept in the dark. The parts of stock plants which have successfully grown in culture are buds, germinating seeds, and intercalary meristems of young leaves. The medium/growth regulator combination which has produced the best growth so far is Linsmaiser and Skoog medium with 5 mg/l 2,4-D. Less contamination occurs when explants are pre-soaked in sucrose and then a fungicide.

Field Trials

Land preparation, planting and management alternatives are first being tested in small replicated field plots. Variables being examined include type of planting material (rhizomes, seedlings, seed), fertilizer application rates, timing of fertilizer application, machine planting and depth of peat removal The most successful techniques will be used to establish larger stands.The objectives of the first three experiments are to determine the effect of fertilizer applications on Typha yields in peat soils, the rate of nutrient uptake over the growing season and the effects of timing the application of fertilizer to coincide with maximum nutrient uptake. Since space limitations preclude the presentation of a detailed description of each experiment, Experiment G8101 will serve as an example.

Experiment G8101 — Fertilization Study

Objectives:

1) Determine the effect of different combinations of fertilizer on the amount of above and below ground biomass produced after one and two years on a previously uncultivated peat soil.
2) Determine the nutrient standing crop after one and two years.
3) Determine the effect of different combinations of fertilizer on density increase and shoot height over the course of two growing seasons.
4) Determine the effect of different combinations of fertilizer on soil fertility after one and two years.
5) Provide a source of above ground biomass for experiments involving harvesting and handling of biomass.

Design:
1) Blocked complete factorial (3 x 2 x 2)
2) Factors: Nitrogen (3 levels), Phosphorus (2 levels), Potassium (2 levels)
3) Levels: a) Nitrogen - 0, 75, and 150 kg (elemental form)
Phosphorus - 0, 150 kg/ha (oxide form)
Potassium - 0, 300 kg/ha (oxide form)
4) Treatments per block: 12
5) Blocks: 4
6) Total Plots: 48
7) Size of Plots: 3 m x 5 m
8) Planting Density: 9 rhizomes/m^2
9) Field Prerparation: a) field was disked 3" deep and dragged, b) 70 kg/ha of Peters Fritted Trace Elements, c) 20 kg/ha of $CuSO_4$, d) plots were fertilized and then rotovated to a depth of 6".

Observations and Sampling:
1) Density
2) Height
3) Insect Damage
4) Competitors
5) Water level
6) Soil samples for nutrient and bulk density analysis
7) Above and below ground plant samples for biomass determination, and tissue nutrient analysis.

REFERENCES

(1) Westlake, D.F.: Comparisons of Plant Productivity. Biol Rev., 38, 385-425 (1963).
(2) van der Valk, A.G. and Davis, C.B.: Primary Production of Prairie Glacial Marshes. In: Good, R.E. et al. (eds.), Freshwater Wetlands: Production Processes and Management Potential, Academic Press, NY, 1978.
(3) Gustafson, T.D.: Production, Photosynthesis and the Storage and Utilization of Reserves in a Natural Stand of Typha latifolia L. Ph.D. Thesis, University of Wisconsin, Madison, 102 pp (1976).
(4) Andrews, N.J and Pratt, D.C.: The Potential of Cattails (Typha spp.) as an Energy Source: Productivity in Managed Stands. J. Minn. Acad. Sci., 44, 5-8 (1978).
(5) Fox, C.A.: Capture of Radiant Energy by Plants. M.S. Thesis, University of Minnesota, 49 pp (1975).
(6) Bray, J.R.: The Chlorophyll Content of Some Native and Managed Plant Communities in Central Minnesota. Can. J. Bot., 38, 313-333 (1960).
(7) Bernard, J.M. and Fitz, M.L.: Seasonal Changes in Above-ground Primary Production and Nutrient Contents in a Central New York Typha glauca Ecosystem. Bull.

Torrey Bot. Club, 106, 37-40 (1978).
(8) Boyd, C.E. and Hess, L.W.: Factors Influencing Shoot Production and Mineral Nutrient Levels in Typha latifolia. Ecology 51, 296-300 (1970).
(9) Kvet, J. and Husak, S.: Primary Data on Biomass Production Estimates in Typical Stands of Fishpond Littoral Plant Communities. In: Dykyjova, D. and Kvet, J. (eds.) Pond Littoral Ecosystems, Springer-Verlag, NY (1978).
(10) Sharma, K.P. and B. Gopal: Studies on Stand Structure and Primary Production in Typha species. Int. J. Ecol. Environ. Sci., 45-66 (1977).
(11) Merezhko, A.I., N.H. Smirnova and V.P. Gorbik: Growth of Stands of Lesser Reedmace (Typha angustifolia) and the Functioning of its Root System. Gidrobiol. ZH., 15, 20-25 (1979).
(12) Mason, C.F. and R.J. Bryant: Production, Nutrient Content, and Decomposition of Phragmites communis Trin. and Typha latifolia L. J. Ecol., 63, 71-96 (1975).
(13) Klopatek, J.M. and Stearns, F.W.: Primary Productivity of Emergent Macrophytes in a Wisconsin Freshwater Marsh Ecosystem. Amer. Midl. Nat., 100, 320-333 (1978).
(14) Van Dyke, G.D.: Aspects Relating to Emergent Vegetation Dynamics in a Deep Marsh, Northcentral Iowa. Ph.D. Thesis, Iowa State University, Ames (1972).
(15) Andrews, N.J.: Unpublished.
(16) Wedin, W.F. and Helsel, Z.: Plant Species for Biomass Production on Managed Sites. Biomass — A Cash Crop for the Future, Kansas City, MO, Proceedings (1977).
(17) Pearsall, W.H. and Gorham, E.: Production Ecology. I. Standing Crops of Natural Vegetation. Oikos, 7, 193-201 (1956).
(18) Gorham, E. and Bernard, J.M.: Midsummer Standing Crops of Wetland Sedge Meadows along a Transect from Forest to Prairie. J. Minn. Acad. Sci. 41, 16-17 (1975).
(19) Bernard, J.M. and MacDonald, J.G.: Primary Production and Life History of Carex lacustris. Can. J. Bot., 52, 117-123 (1974).
(20) Bernard, J.M. and Solsky, B.A.: Nutrient Cycling in a Carex lacustris Wetland. Can. J. Bot., 55, 630-638 (1970).
(21) Moss, D.N.: Improvement of Plant Photosynthesis through Genetic Engineering. Symposium Papers, Clean Fuels from Biomass and Wastes, I.G.T., 63-71 (1977).
(22) Pratt, D.C., Bonnewell, V., Andrews, N.J. and Kim, J.: The Potential of Cattails as an Energy Source. Final Report to the Minnesota Energy Agency (1980).
(23) Dykyjova, D., Veber, K. and Priban, K.: Productivity and Root/Shoot Ratio of Reedswamp Species Growing in Outdoor Hydroponic Cultures. Folio Geobot. Phytotax., Praha, 6 (2), 233-254 (1971).
(24) White, J.M. and Sinclair, L.R.: Effect of Plant Spacing on Growth and Yield of Transplanted Cattails. Proc. Soil Crop Sci. Soc. Fla., 38, 18-20 (1979).
(25) Andrews, N.J., Penko, M., Mattson, M.D., and Pratt, D.C.: The Establishment of Cattails on a Minnesota Peatland. Report to the Minn. Department of Natural Resources, 100 pp.
(26) Bonnewell, V.: Typha Productivity, Mineral Nutrition, and Seed Germination. Ph.D. Thesis, University of Minnesota, 132 pp (1981).