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Research, Development, and Demonstration of Algal Production Raceway (APR) Systems for the Production of Hydrocarbon Resources

A Subcontract Report

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FOREWORD

This report is the final report for FY 1983. The work was performed under subcontract to SERI with funds provided by the Biomass Energy Technology Division of the U.S. Department of Energy, under a program investigating the mass culture of microalgae as a source of renewable fuels.

Robins P. McIntosh, Manager
Aquatic Species Program

SUMMARY

Objective

To determine the conditions and techniques appropriate for maximizing lipid yield from halophilic microalgae and to quantify the performance and operational parameters for a shallow raceway system.

Discussion

A shallow raceway system has been developed and constructed by the University of Hawaii. Briefly, the system concept includes shallow channels circulated at a high flow rate, containing very dense algal cultures. The entire system was covered with a bath containing a copper sulfate solution, which served to absorb incident infrared radiation and perhaps to stimulate lipid production. It was proposed that the heat accumulated by the copper sulfate solution could be utilized to power the entire system. The prototype system was enclosed on all sides to minimize contamination.

The Hawaii system was constructed with an area of 50 m² and incorporated most, but not all, of the original design features. Airlift pumps, restricted within 6-in. PVC pipes rather than open as in the IFP system, were used for recirculation and gas exchange. The system was covered with panels containing a 1-cm-thick layer of a 5% copper sulfate solution, suspended at about 30 cm over the surface of the culture and slightly tilted to the south to correct for sun angle and to permit rainwater runoff. Cultures were grown at a depth of 7.5 cm and circulated at a velocity of about 25 cm/sec. The cultures were not fully enclosed and, after initial testing, the copper sulfate panels were removed. The panels were effective as infrared shields, but their overall effect on production was judged to be detrimental because they also removed about 50% of the photosynthetically active radiation (400-700 nm wavelength). The net negative effect of these panels on production was later confirmed by factorial experiments in four smaller cultures of similar design, each with 9.2 m² of surface area, constructed in 1982 and 1983 at the same site. The same experiments confirmed, however, that the panels made a significant contribution to temperature control in the cultures.

A major contribution of the Hawaii project has been the development of a system that introduces ordered vertical mixing into the culture as a by-product of stream flow. Small foils, similar in design to airplane wings, were suspended in the flowstream at a relatively high angle of attack. Vortices were generated at the tips of each of these foils and were propagated downstream. Arrays of foils were positioned every 1.2 m along the culture flume. Observations of the vortex rotation rates indicated that cells were exposed to a light-dark cycle time of 1 to 2 seconds in this mixing regime,

compared to the 10 seconds predicted for a purely turbulent regime. In addition, light-dark cycling was provided in a patterned manner rather than on a random basis. Productivity in a system employing the foils was increased by a factor of 2.2 over the productivity in the same system without the foils, but again it is not completely clear that light modulation was solely responsible for the increased productivity.

Conclusions

A fractional factorial experimental design was used to determine the maximum production and photosynthetic efficiency that could be achieved in shallow algal mass culture systems (SAMCS) of the marine diatom Phaeodactylum tricornutum. Dilution rate and CO₂ supply were found to be the most important system parameters. Maximum production was found to be about 25 g dry wt m⁻²d⁻¹. This production corresponded to a photosynthetic efficiency of 5.6%. These figures are 50-100% better than the production rates achieved in earlier P. tricornutum cultures using conventional culture techniques. The results are consistent with a theoretical model of the impact of the flashing light effect on algal mass culture production. This model predicts that at the typical irradiances in Hawaii, full utilization of the flashing light effect should enhance production by 70% to over 200%. It was concluded that the use of foil arrays in the experimental flume creates systematic vertical mixing on a time scale suitable for utilizing the flashing light effect. Harvesting P. tricornutum cells is accomplished straightforwardly by adding approximately 0.5 mM NaOH to the culture. With this addition the cells rapidly flocculate and settle out with almost 100% efficiency. Production of P. tricornutum culture is probably limited by temperature. P. tricornutum cannot survive at temperatures in excess of 25°C in outdoor mass cultures. Growth of mesophilic species in the temperature range 30-35°C may well result in even higher production than that achieved with P. tricornutum.

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SECTION 1.0

INTRODUCTION

1.1 HISTORICAL BACKGROUND

Microalgae are attractive as biomass producers because they generally exhibit higher yields and photosynthetic efficiencies than terrestrial plants. In addition, many species of microalgae produce high concentrations of intracellular oils as energy storage compounds. Microalgae are projected from laboratory scale experiments to be capable of producing over 100 barrels of liquid fuel per acre-year. Most pilot-scale mass culture systems, operated at depths of greater than 20 cm, have shown peak average yields of around 15 dry ash free grams $m^{-2}d^{-1}$. This project is based on the premise that operation at less than 15 cm depth will result in significantly higher productivities.

Research on this problem began at the University of Hawaii under SERI subcontract on February 15, 1980 under the title "Research, Development, and Demonstration of Algal Production Raceway (APR) Systems for the Production of Hydrocarbon Resources." Work during the first two and one-half years resulted in the collection of a great deal of laboratory data on the performance of Phaeodactylum tricornutum; the design, construction, and operation of one $48m^2$ continuous flume; the development of predator control techniques and baseline performance characteristics for the flume; and the construction of four $9.2m^2$ experimental flumes. During the past year these four flumes were operated in a factorial design experiment to determine the maximum sustainable biomass yields achievable with P. tricornutum cultured in shallow flume systems. Secondary goals of the past year's work were to 1) gain an understanding of the mechanisms and conditions which lead to high productivity, photosynthetic efficiency, and lipid yield, and 2) analyze the performance in response to operational improvements projected to arise from further research and development. It was expected that these goals would be achieved through work on the following four tasks

- I Operational Parameter Screening
- II Yield Optimization
- III Analysis, Modeling and Laboratory Support Studies
- IV Performance Sensitivity Analysis

1.2 THEORETICAL BACKGROUND

It has been known for a number of years that the efficiency of light utilization by phytoplankton can be markedly increased by exposing the cells to alternating periods of light and dark (1-7). More recently, a number of investigators have explored this

phenomenon both experimentally and theoretically (8-15). Although early work (2-4) indicated that flash periods as short as 10 μ s were needed to take maximum advantage of the flashing light effect, it is now clear that flash periods as long as 10-70 ms may still result in significant enhancements of photosynthetic efficiencies (14,16), and Marra (13) has reported increases up to 87% in photosynthesis by simply modulating the irradiance on algal cells on a time scale ranging from minutes to hours. It seems reasonable to postulate that the physiological mechanisms responsible for these observed photosynthetic enhancements differ substantially over the vast range of time scales involved. However, regardless of the physiological mechanisms involved, it is clear that photosynthetic efficiencies may be enhanced by a factor of two or more by modulating or flashing the incident light on an appropriate time scale. For the case of flashing light, it is apparent that the maximum duration of the light pulse which produces the highest photosynthetic efficiency will be negatively correlated with light intensity at light intensities greater than or equal to I_K , the light intensity above which photosynthetic efficiency begins to decrease under constant illumination (17), and that the duration of the dark period must lie somewhere in the approximate range 0.1-10s (18).

A critical question in algal mass culture work is whether a physical mechanism exists for taking advantage of the flashing light effect in a practical way. As noted by Phillips and Myers (14), "Any attempt to grow algae in sunlight will experience some gain by turbulence. The feasibility of increasing the turbulence will depend upon the extent of the gain in growth as compared to the increased power requirements of stirring or pumping the suspension." Unfortunately, it does not appear that merely producing random turbulence is sufficient to take advantage of the flashing light effect to any significant degree in a mass culture system. Powell, Chaddock, and Dixon (15) reached this conclusion based on theoretical calculations, and Miller et al. (19) commented that, "Utilization of the flashing light effect for improvement of photosynthetic efficiency of dense algal cultures requires a nonrandom mixing pattern-one in which cells are exposed to regular sequences of light and darkness..."

In the past two types of experimental systems have been studied as possible means of utilizing the flashing light effect. A so-called Couette device was used by Miller et al. (19), Davis et al. (20), and Howell, Fredrickson, and Tsuchiya (21). The Couette device consists of two concentric, circular cylinders, the inner of which can be rotated at a selected speed. The system is illuminated either from the outside or inside, and the culture is grown in the gap between the cylinders. The flow pattern in the culture medium is complex, but sufficiently nonrandom that a systematic flashing light effect is achieved. Howell, Fredrickson, and Tsuchiya (21) estimated the period of the light-dark cycles in their system to be about 40 msec. More recently, Oswald et al. (22)

and Shelef, Sabanas, and Oswald (23) have described a chemostat-type system called an alga-tron, which consists of a drum that is rotated about its vertical axis. The algal culture is contained within the drum as a film a few centimeters thick on the inside wall of the drum, and is retained against the wall by centrifugal force. A light source is mounted inside the drum, and a row of steel strips extending to within 0.5-1.0mm of the inside wall produces highly turbulent wakes, which extend in spirals around the culture. The photosynthetic efficiencies which can be achieved with the Couette device and alga-tron are on the order of 10% (17).

It seems unlikely that either the Couette device or the alga-tron will prove to be a practical means of utilizing the flashing light effect. Both devices are mechanically complex, and the power requirements for operating them may well offset any increase in production, both from a financial and an energetic standpoint. As noted by Fredrickson and Tsuchiya (17), "Clearly some device that is mechanically more simple than the foregoing devices is required." The mechanism we have devised to produce systematic vertical mixing in our flume is illustrated in Figures 1-1 and 1-2. The device consists of a series of foils similar in design to airplane wings placed across the flume. As water flows over and under these foils, a pressure difference is created as illustrated in Figure 1-1. At the tips of the foil, the flow of water from the high pressure region below the foil to the low pressure region above the foil creates a vortex off each tip of the foil. If the foils are properly spaced along a suitable supporting structure (Figure 1-2), the vortices on adjacent foils rotate in opposite directions and thus reinforce each other. The width of each foil and the gap between foils are equal to the depth of the culture, so that circular vortices created by the foils effectively mix the culture from top to bottom (Figure 1-2). The system of vortices with rotational axes parallel to the direction of flow produces the sort of systematic mixing necessary to produce the flashing light effect.

SECTION 2.0

MATERIALS AND METHODS

2.1 THE CULTURE SYSTEM

The flumes utilized in our studies consisted of one 48m² continuous flume and four 9.2m² continuous flumes. The 48m² flume is illustrated in Figure 2-1, and specifications for the 48m² flume appear in Table 2-1. The flume is of a modular construction. The base channel module is a 1.2x2.4m molded fiberglass trough with 10 cm sides. The sides are raised an additional 23 cm with a plywood extension, coated with acrylic resin (Figure 2-2). All seams are covered with fiberglass cloth and resin. The channel

 Flume Specifications

Construction	Modular: 1 liftbox module 1 turning box/heat exchanger 1 drainbox 14 channel modules 3 recirculation pipes/airlifts
Centerline channel length	37.2 m
Area	48.39m ²
Depth range	5-28 cm
Operating depth	7.75 cm (standard deviation 0.74 cm, 91 measurements), with upper level electrode set at 7.6 cm.
Volume at operating depth	4150 L
Flow rate	30 cm/s (circuit time 120 s)
Slope	0.16 cm/m

Module Specifications

Liftbox	
Construction	2-cm plywood painted with fiberglass resin
Area	1.49 m ²
Volume at operating depth	115 L
Turning box/heat exchanger	
Construction	2-cm plywood painted with fiberglass resin 3.2 tubular titanium PVC fittings
Area	3.73 m ²
Volume at operating depth	290 L
Drainbox	
Construction	2-cm plywood painted with epoxy resin
Area	1.49 m ²
Volume at operating depth	220 L
Channel modules	
Construction	Spray-molded fiberglass PVC reinforcements 2-cm plywood painted with fiberglass resin
Area	2.98 m ² each module, 41.67 m ² total
Volume at operating depth	230 L each module, 3225 L total
Recirculation	
Construction	15 cm i.d. PVC pipe PVC fittings epoxy resin silicone glue (three separate pipes, nested, with separate airlifts)
Volume:	300 L
Airlift pumping rate:	690 liter min ⁻¹ each lift, 2070 liter min ⁻¹ total
Airlift life height:	163 cm (air outlet to water surface at operating depth)
Airlift air consumption rate:	550 liters min ⁻¹

Figure 1-1. Design of a single foil indicating mechanism of vortex production.

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Figure 1-2. Positioning of individual foils in foil array. Lower figure indicates positioning of array in the flume. D is the depth of the water. Arrays indicate rotational direction of vortices.

Figure 2-1. Perspective to algal production flume showing location of heat exchanger, lift box, drain box, and airlift system.

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Figure 2-2. Cross section of flume modules indicating positioning of chill pipes, airlift, and CO₂ feed.

modules are reinforced with split 7.5 cm diameter polyvinyl chloride (PVC) pipe, and are mounted on 5x10 cm wooden rails supported by concrete blocks.

In addition to the channel modules, there are two end-box modules and a heat exchanger module which also serves as a turning box. One end-box serves as a drain box, the other as a lift box, and they are connected by three recirculation pipes with airlifts. The drain box (Figure 2-2) includes a small 15-cm deep sump to provide for even flow. The airlifts discharge directly onto the level surface of the flume in the lift box (Figure 2-2). The recirculation pipes (Figure 2-3) are 15 cm outside diameter PVC pipe turned in a U-shape using 45° PVC elbows to reduce flow resistance over that which would be introduced by 90° elbows. Each of the two inner recirculation pipes has a small drain port at the bottom of the U; there is a 15-cm valve and drain line located in the outermost recirculation pipe which allows the culture to be transferred to the culture storage tank (Figure 2-4). The airlifts are fed by 2.4 cm PVC pipes which discharge 1.4 m below the water surface. Oil-free air is supplied by a water-sealed, water lubricated compressor driven by a 3.5 horsepower motor. The heat exchanger consists of twenty-two 2.5 cm titanium pipes mounted in the 1.2x3 m turning box (Figure 2-5). When cooling is required, cold water is circulated through the titanium pipes from the chiller. Activation of the chiller is maintained under computer control.

A seawater system (Figure 2-4) provides capabilities for adding seawater to the flume, obtaining seawater for laboratory use, partially or completely draining the flume, and storing the culture or seawater. Seawater is drawn from a 45-m-deep well. The seawater is filtered through coral rubble, and is therefore very low in particulate organic matter. Levels of major inorganic nutrients are typically 50 μM ammonium and 0.8 μM phosphate. The inlet to the seawater pump is from the saltwater well or from the storage tanks. The outlet is to the flume, to the utility outlet, or to waste. The flume can be drained into the culture storage tank through the 15-cm PVC pipe using a manual valve, and pumped from that tank to any of the outlet ports, or discarded by gravity through the waste port. Wastewater is discharged into an underground sump excavated in the coral rubble.

The flume incorporates a computer-based system that monitors and controls the physical and chemical environment of the culture. The system controller is a Hewlett-Packard model 9845B computer, which acquires data from the sensors and manipulates the control devices based on the information received. The chiller compressors, fans, and pump are controlled on the basis of the culture temperature. The seawater pump is controlled on the basis of the operator input and the output of the level detector. Carbon dioxide gas addition is controlled on the basis of pH. Draining is controlled on a time-of-day basis. The addition of a base (0.6M NaOH) is controlled on the basis of pH.

Figure 2-3. Detail of recirculation and airlift system.

All dimensions in centimeters.

Figure 2-4. Schematic of seawater supply system and algal storage tank.

Figure 2-5. Overhead perspective of flume indicating horizontal dimensions and positioning of CO₂ feed and airlifts.

An additional, computer-independent device is also provided to protect the culture from overheating in the event of computer failure. This over-temperature control prevents the flume temperature from rising above 30°C by independently activating the chillers.

The four 9.2m² flumes are similar in design to the 48m² flume, but consist of two channels 7.6 m long by 0.6 m wide by 0.15 m deep. The channels are connected by 20 cm recirculation pipes; one of the two recirculation pipes of each culture contains an airlift pump which drives culture circulation. Each 9.2m² flume is equipped with a CO₂ supply line and with a 1.5 horsepower chilling unit, which exchanges heat with the water through 6 m of 2.5 cm diameter titanium pipe. The titanium heat exchangers are U-shaped and are positioned just upstream of the downflow to one of the recirculation pipes. Temperature and pH sensors in the cultures supply data to the Hewlett-Packard 9845B computer, which in turn controls chiller function and the CO₂ supply solenoids.

The experimental results reported here were all obtained with the marine diatom Phaeodactylum tricornutum. This species was chosen as a test organism because of its known tendency to dominate outdoor mass cultures (24), its wide range of tolerance to pH, salinity and temperature (25-28), its ability to withstand buffeting (29), and the fact that excretory products produced by P. tricornutum appear to be inhibitory to the growth of bacteria as well as other algae (25,30). The particular strain of P. tricornutum used in these studies was obtained from the culture collection of Dr. William Thomas at the Scripps Institute of Oceanography.

2.2 ANALYTICAL DATA

Production in the flume was calculated from daily changes in particulate carbon (PC) concentrations corrected for dilution due to drainage and introduction of fresh medium. Samples for PC analyses were collected on glass-fiber (GF/C) filters and analyzed on a Hewlett-Packard model 185B CHN analyzer following procedures recommended by Sharp (31). These carbon production numbers were converted to dry weight production by multiplying by the experimentally determined ratio of dry weight:PC. We found this ratio to be 2.0. Dry weights were measured by filtering samples onto GF/C filters and drying at 60°C to constant weight. The percent ash was calculated from samples also collected on GF/C filters, and combusted at 500°C for four hours. We found that ash accounted for less than 5% of the dry weight of P. tricornutum when no silicate additions were made to the culture medium. Lipid contents were measured by a modified Bligh-Dyer extraction. Culture NH₄⁺ concentrations were determined by the phenol-hypochlorite method. Urea concentrations were determined by the same method after the samples were digested with urease for 15 min.

Solar irradiance was measured with a Lambda model LI-1905B quantum

sensor, which has a nearly flat response to light in the wavelength range 400-700 nm. Visible quantum fluxes were converted to energy fluxes using the spectral distribution of sunlight (32). The conversion ratio was 19.5 μ Einst per calorie. The caloric content of the product algae was calculated assuming a heat of combustion of 6225 cal/g dry wt (29), and solar energy conversion efficiencies were calculated based on this caloric content and the incident energy flux of visible light.

SECTION 3.0

RESULTS

3.1 TASK I: OPERATIONAL PARAMETER SCREENING

Objective: • To perform blocked fractional factorial biomass yield experiments on eight operational parameters using four 9.2m² shallow algal mass culture systems (acronym SAMCS)

- To rank main effects and interaction effects of parameters with regard to impact on photosynthetic efficiency and biomass yield

Purpose • To reduce the number of parameters which must be studied in detail in the optimization effort (Task II).

The fractional factorial design employed is shown in Table 3-1, and is given in terms of the factor values in Table 3-2. In Table 3-1, each variable is assigned the + value in eight runs and the - value in eight runs. For the eight + values or the eight - values for each factor, each other factor assumes four + and four - values. Thus the effects of all other factors, if additive, will cancel in the calculation of the primary effects of a given factor.

Details of the manipulation of each experimental factor are as follows:

1. Depths were 5 cm or 10 cm mean depth. Depths varied as a function of the distance along the channel, with ranges up to ± 2 cm about the mean. Six sets of mixing foils were placed in each culture spaced at 1.5 m intervals to provide ordered mixing. Foil design was depth dependent: at the 5 cm depth the foils were 5 cm wide with 5 cm gaps between, while at the 10 cm depth these dimensions were 10 cm.
2. Dilution rates are reported as the percentage of the culture removed each morning and replaced with fresh medium. Due to errors in measuring depth, dilution rates varied about the desired means.
3. Temperatures reported are the temperatures above which the

Table 3-1. A fractional factorial experimental design for eight factors in
16 runs (2_{IV}^{8-4}).

RUN	LEVEL FOR FACTOR:							
	1	2	3	4	5	6	7	8
1	-	-	+	-	-	+	-	+
2	+	-	-	-	+	-	-	+
3	+	+	+	+	-	-	-	+
4	-	+	-	+	+	+	-	+
5	+	-	+	-	-	-	+	-
6	-	-	-	-	+	+	+	-
7	-	+	+	+	-	+	+	-
8	+	+	-	+	+	-	+	-
9	+	+	-	-	-	+	+	+
10	-	+	+	-	+	-	+	+
11	-	-	-	+	-	-	+	+
12	+	-	+	+	+	+	+	+
13	-	+	-	-	-	-	-	-
14	+	+	+	-	+	+	-	-
15	+	-	-	+	-	+	-	-
16	-	-	+	+	+	-	-	-

Table 3-2.

Raceway Experimentation Schedule -- Fractional Factorial Analysis

	Raceway	1 Depth	2 Dilution rate	3 Night T°C ²	4 Flow Rate	5 CuSO ₄	6 pH ³	7 Nutrient	8 Salinity
	#	cm	(d ⁻¹)		cm/s				0/00
BLOCK #1	1	5	20	20	15	-	9.0	NH ₄ ⁺	35
	2	10	20	15	15	+	8.0	NH ₄ ⁺	35
	3	10	40	20	30	-	8.0	NH ₄ ⁺	35
	4	5	40	15	30	+	9.0	NH ₄ ⁺	35
BLOCK #2	1	10	20	20	15	-	8.0	urea	15
	2	5	20	15	15	+	9.0	urea	15
	3	5	40	20	30	-	9.0	urea	15
	4	10	40	15	30	+	8.0	urea	15
BLOCK #3	1	10	40	15	15	-	9.0	urea	35
	2	5	40	20	15	+	8.0	urea	35
	3	5	20	15	30	-	8.0	urea	35
	4	10	20	20	30	+	9.0	urea	35
BLOCK #4	1	5	40	15	15	-	8.0	NH ₄ ⁺	15
	2	10	40	20	15	+	9.0	NH ₄ ⁺	15
	3	10	20	15	30	-	9.0	NH ₄ ⁺	15
	4	5	20	20	30	+	8.0	NH ₄ ⁺	15

1 - after dilution

2 - compressor cut-in

3 - CO₂ cut-in

chiller for each culture was activated. Average temperatures in the cultures differed between the two treatments by 2-4°C. Variables other than chiller activation temperature also affected the mean culture temperature (see following discussion).

4. Flow rates were 15 and 30 cm sec⁻¹, and were adjusted by varying the volume of air supplied to the airlift pump.
5. Blue light was introduced by placing double-walled Plexiglas sheets filled with 5% CuSO₄ solution above the cultures. The average light path through the CuSO₄ solution was about 1 cm.
6. The pH factor denotes the culture pH above which the CO₂ supply solenoid was activated to supply approximately 200 ml min⁻¹ of 100% CO₂ gas.
7. Nitrogen nutrient sources were ammonium or urea, both supplied as commercial fertilizers. Concentrations were maintained at 0.5 - 1.5 mM N. Samples for NH₄⁺ and urea concentrations were taken daily. If visible comparison to a standard solution showed concentrations of 0.5 to 1.0 mM, sufficient nutrient was added to cause a 0.5 mM increase. If concentrations were less than 0.5 mM, enough nutrient was added to cause a 1.0 mM increase. Equimolar (N:C) amounts of sodium bicarbonate were added along with the ammonium sulfate to offset alkalinity changes due to ammonium uptake.
8. Salinity was altered by diluting the 35-40 ‰ well water with tap water to achieve 15 ‰. For 35 ‰, the well water was undiluted.

Since all 16 runs could not be executed simultaneously, "blocking" of the factorial design was employed to divide the 16 runs into four sets of four runs each (Table 3-2). In such a blocked design there can be variability due to changes in uncontrolled variables between blocks. Effects of such variation cannot be distinguished from the effects of factors which have consistent signs within blocks. Table 3-3 shows the primary effects and two-factor interactions which are confounded with block effects. The table also shows the differences between the mean values for the + blocks and the mean value for the - blocks of the measured environmental variables air temperature and daily irradiance. If either of these variables, but particularly light intensity, significantly affect culture performance, substantial block effects would be expected. The block effects will be confounded with the effects of factors 7 and 8. Unmeasured variables such as well water quality could also have affected the cultures and have led to block effects.

Table 3-3. Block effects of measured environmental variables.

FACTOR	<u>SIGN IN BLOCK</u>				<u>TEMPERATURE EFFECT</u>	<u>LIGHT EFFECT</u>
	1	2	3	4	°C	$E\ m^{-2}\ day^{-1}$
7	-	+	+	-	-0.25	-0.35
8	+	-	+	-	+0.35	+3.65
16,35,78	-	-	+	+	+0.35	+13.25
24	+	+	-	-	-0.35	-13.25

3.2 FRACTIONAL FACTORIAL ANALYSIS - SAMPLE RESPONSE

Table 3-4 lists the 22 distinguishable effects from the fractional factorial design employed; while there were 36 total primary and two-factor effects, 21 of the second order effects were confounded in groups of three, leaving $15+7 = 22$ distinguishable effects. [Second order effects are referred to here by combinations of the digits referring to the factors involved; i.e. 24 is the second order effect involving dilution rate (2) and flow rate (4)]. Also, for each set of three confounded two-factor effects, there is one other two-factor effect which differs only with respect to sign (Table 3-4).

Only primary and two-factor effects were calculated; normally, higher order effects become progressively smaller, and can be ignored. Primary effects were calculated as

$$E(I) = \sum_{n=1}^{16} R(n) \cdot \text{Sign}(I,n) \quad (1)$$

where $E(I)$ is the effect due to factor I , $R(n)$ is the measured response in run n , and $\text{Sign}(I,n)$ is the sign of factor I in run n (+1 or -1). Two-factor effects were calculated as

$$E(I,J) = \sum_{n=1}^{16} R(n) \cdot \text{Sign}(I,n) \cdot \text{Sign}(J,n) \quad (2)$$

To demonstrate the data analysis technique in an idealized case, we have selected a culture response which should be affected by only certain of the factors employed. The example presented here is culture temperature; other examples can be found in Table 3-5. The effects calculated according to equations 1 and 2 will ideally be distributed normally about their mean if all effects are random. The cumulative frequency distribution of these effects, when plotted on probability paper, should appear linear. Any effects at the tails of these curves which fall outside of this linear relationship are thus greater than would be predicted by probability. In the case of temperature, two factors had considerable effects on culture temperature, 3 (chiller cut-in) and 5 (CuSO_4 panels)(Figure 3-1). These are, in fact, the variables which we would most expect to influence culture temperature. None of the other variables, with the possible exception of 1 (culture depth, hence volume) would be expected to significantly affect temperature. [Factor 1 was in fact the next most significant variable (Table 3-5)].

3.3 FRACTIONAL FACTORIAL ANALYSIS - BIOLOGICAL EFFECTS

The effects of the various factors on biomass and lipid production

Table 3-4. Confounding of effects in the fractional factorial analysis.

<u>EFFECT</u>	<u>EQUIVALENT TO:</u>
1	
2	
3	
4	
5	
6	
7	BLOCK
8	BLOCK
12	(-37, -46, -58)
13	48, 56 (-27)
14	38, 57 (-27)
15	36, 47 (-28)
16	35, 78 (-24) BLOCK
17	45, 68 (-23)
18	34, 67 (-25)
23	(-17, -45, -68)
24	(-16, -35, -78) BLOCK
25	(-18, -34, -67)
26	(-14, -38, -57)
27	(-13, -48, -56)
28	(-15, -36, -47)
37	46, 58 (-12)

Table 3-5. Summary of factor effects on various culture responses. Effects are given in standard deviations.

RESPONSE	<u>1</u>	<u>2</u>	<u>3</u>	<u>4</u>	<u>5</u>	<u>6</u>	<u>7</u>	<u>8</u>
1. Biomass Production	2.12	-0.86	0.80	0.60	-0.51	-0.99	-0.66	-0.14
2. Photosynthetic Efficiency	2.13	-0.74	0.76	0.41	-0.57	-1.28	-0.39	-0.51
3. Lipid Production	1.91	-1.14	0.76	0.03	0.09	-0.77	0.36	0.11
4. Particulate Carbon Concentration	-0.58	-1.95	0.52	0.36	-0.04	-1.03	-0.69	2.48
	0.68	-0.53	2.39	0.49	-3.42	-0.11	0.12	0.44
5. Temperature	0.12	0.04	-0.14	-0.50	-1.61	2.31	-0.81	-1.54
6. pH	-0.69	-0.38	0.47	1.65	1.14	-0.58	-1.26	-2.33
7. Salinity

Figure 3-1. Normal plot of factor effects on observed culture temperature.

by the cultures, as well as other biological parameters, were not so simple to distinguish. Figure 3-2 shows factor effects on culture carbon production ($\text{gC m}^{-2}\text{d}^{-1}$). No effect, with the possible exception of 1 (depth), lies outside the predictions of probability. The second largest single factor effect was due to salinity (factor 8), but this factor is confounded with block effects, as are the largest two-factor interactions, 16 and 24. Photosynthetic efficiency effects, calculated on the basis of carbon production, showed a particularly tight linear relationship on a normal plot (Figure 3-3). Not surprisingly, factor 1 and the block effects 8, 16, and 24 were also the most significant.

Although none of the biological effects showed significantly non-normal distributions, it does not necessarily follow that the calculated effects do not represent real responses to the variable factors. The significance of the effects could be better evaluated if the variability of repeated runs under constant conditions were known. In the absence of such data, we took the standard deviation of the observed effects as a maximum estimate of the variability of the repeated runs, and normalized the effects to this standard deviation (Table 3-5). Based on the data in Table 3-5, we have listed in Table 3-6 the most important factors affecting biomass production, lipid production and photosynthetic efficiency.

By far the most significant factor affecting production and photosynthetic efficiency was culture depth. Deeper cultures showed greater production, because self-shading effects were more than offset by higher areal standing crops. Higher areal standing crops might also be achieved by increased culture density, but the latter variable is not always under the control of the culture operator. The second most important factor affecting biomass production and photosynthetic efficiency was pH (CO_2 supply cut-in); cultures which did not receive CO_2 until the pH reached 9 showed less production. Increased dilution caused decreased production, because the standing crop was reduced to too low a level. Increased temperature gave increased production, an expected result if the temperature range of the species is not exceeded.

3.4 SELECTION OF VARIABLES FOR FURTHER STUDY

Depth, and thus areal culture density, was the most clearly significant factor in determining production. Since our 9.2m^2 flumes were designed to be operated at a maximum depth of 10 cm, we elected to conduct further experiments at that depth. The 48m^2 flume is the only facility available to us which could be used to study the effect of changes in culture depth at depths much greater than 10 cm. We are skeptical that the use of foils to effect systematic vertical mixing would be very useful in cultures much over 10 cm deep. In any case we felt that experimentation with culture depths greater than 10 cm was best postponed for later work with the 48m^2 flume, since conducting such experiments in the

Figure 3-2. Normal plot of factor effects on carbon production.

Figure 3-3. Normal plot of factor effects on photosynthetic efficiency.

Table 3.6 Most important factors affecting production and photosynthetic efficiency.

Response	Factor	Effect (s.d.'s)
BIOMASS PRODUCTION	Depth	+2.13
	pH (CO ₂ cut-in)	-1.13
	Dilution Rate	-0.80
	Temperature (chiller cut-in)	+0.78
LIPID PRODUCTION	Depth	+1.91
	Dilution Rate	-1.14
	pH (CO ₂ cut-in)	-0.77
	Temperature (chiller cut-in)	+0.76
PHOTOSYNTHETIC EFFICIENCY	Depth	+2.13
	pH	-1.28
	Temperature (chiller cut-in)	+0.76
	Dilution Rate	-0.74

9.2m² flumes would require major modifications to raise the sides.

Previous work (24,25) has made it clear that the ability of P. tricornutum to dominate outdoor mass cultures diminishes rapidly as the temperature is raised from 20 to 25°C. Our results indicated a positive correlation between production and temperature in the range 15-20°C, but because of the difficulty in maintaining P. tricornutum cultures at temperatures a few degrees above 20°C, we felt it was reasonable to establish the operating temperature at 20°C.

With culture depth and temperature thus established at levels at or very close to optimum values, we concluded that pH and dilution rate were the most important variables for further study. Flow rate had a positive effect on production and efficiency (Table 3-5), and was therefore set at 30 cm sec⁻¹ for further studies. This flow rate is about the maximum we can achieve in our present flumes. Due to the negative effect of the copper sulfate panels on production and photosynthetic efficiency, we chose not to apply these panels in the optimization experiments. The results in Table 3-5 indicate that ammonium (-sign in factorial) was superior to urea (+ sign in factorial) as a nitrogen source, but it is debatable whether the difference is statistically significant. To be on the safe side, we decided to use NH₄⁺ in further studies. Production and photosynthetic efficiency were greater at 15 ‰ (- sign in factorial) than at 35 ‰ (+ sign in factorial). The effect was of debatable statistical significance, but again to be on the safe side we chose to conduct further experiments at 15‰.

3.5 TASK II: YIELD OPTIMIZATION

Objective: ● To determine how much P. tricornutum production can be obtained from a SAMCS

- To determine the sensitivity of the production function to changes in design characteristics

Purpose ● To determine whether SAMCS are significantly more productive than conventional algal mass culture systems

Based on the results obtained in Task I, we elected to perform a two-way, first-order factorial design experiment to determine the local slope of the response surface, and in particular to determine the equation for the path of steepest ascent to the approximate location of the production peak. Dilution rate and pH were the two parameters to be varied. Our Task I results had indicated that both pH and dilution rate were negatively correlated with production in the pH range 8-9 and the dilution rate range 20-40%. However, a surprising result emerged when we began to explore the response function at pH values less than 8. Although we continued to find a negative correlation between production

and pH, the correlation between production and dilution rate was now positive. The approximate equation for the path of steepest ascent was

$$\text{Dilution Rate} = 484 - 58 \text{ pH} \quad (3)$$

The explanation for this relationship is apparent in Figure 3-4. The percentage of inorganic carbon present as CO_2 in water dramatically increases as the pH is lowered below 8. At pH values between 8 and 9, almost all the inorganic carbon is bicarbonate. Thus varying the pH between 8 and 9 has relatively little influence on the availability of CO_2 . The implication of our results is that in order to produce high growth rates in a dense culture of P. tricornutum, one must not only add supplemental inorganic carbon, but must also insure that more than a small percentage is present as CO_2 rather than bicarbonate.

One additional piece of information guided our search for the region of the production peak. We knew from previous work that the maximum growth rate of P. tricornutum corresponded to almost exactly a tripling of the biomass in one day (33). Since growth at the maximum growth rate implies no light limitation, it is reasonable to conclude that maximum areal production would be achieved if the culture were diluted once each day by less than 67%. Equation 3 implies that the pH corresponding to a dilution rate of 67% per day would be about 7.2. For our second-order factorial design, we elected to explore the response function in the pH range 7.2 to 8.4 and the dilution rate range 30-60% per day.

The results of this analysis are shown in Figure 3-5. Production was positively correlated with dilution rate for $30\% \leq \mu \leq 50\%$, but production at dilution rates of 50% and 60% was essentially the same. The optimum pH was estimated to be 7.0 - 7.5, and the optimum dilution rate 50-60%. The curves drawn in Figure 3-5 were obtained by expanding the response function (production) in a power series of the form

$$P(\text{gC m}^{-2} \text{d}^{-1}) = C_0 + C_1 \mu + C_2 \text{ pH} + C_3 \mu^2 + C_4 \text{ pH}^2 + C_5 \mu \text{ pH} + C_6 \mu^4 \quad (4)$$

where μ is dilution rate and the C_i are constants. A careful examination of the data suggests that this truncated expansion probably creates one misleading impression. The curves show a positive correlation between pH and production at the lower end of the pH range. Actually our data show no evidence of such a positive correlation; we suspect that between a pH of 7 and the optimum pH at each μ there is very little correlation between pH and production. However, forcing the pH to a value less than the optimal value would waste CO_2 . Therefore it seems reasonable to operate the flume at the optimum pH implied by the curves in Figure 3-5, and to keep the dilution rate in the range 50-60%.

Figure 3-4. Percentages of dissolved carbon dioxide, bicarbonate ion and carbonate ion as a function of pH at 0°C. Continuous line, distilled water; broken line sea water.

Figure 3-5. Production of carbon in experimental flumes as a function of pH and dilution rate. Curved lines are equation 4.

The least squares fit of Equation 4 to our data indicates that maximum production of about 26g dry wt $m^{-2}d^{-1}$ is achieved at a pH of 7.25 and at $\mu = 57.6\%$. Production is predicted to drop by about 30% if μ is reduced to 40% per day, and by roughly another 30% if μ is reduced to 30% per day. Production is fairly sensitive to pH for pH values above the optimum. For example, at $\mu = 40\%$, production drops by about a factor of two as pH is increased from 7.6 to 8.4 (Figure 3-5). However, at pH values below or near the optimum pH, production is rather insensitive to changes in pH. This fact is apparent from the observation that production at $\mu = 30\%$ was almost independent of pH for pH values between 7.7 and 8.5.

Probably the most complete and up-to-date summaries of algal mass culture production figures are those of Goldman (34) and Aaronson and Dubinsky (35). The highest yields that have been achieved with P. tricornutum are 9.8 g dry wt $m^{-2}d^{-1}$ by Ansell et al. (36) during May-August in England and 12.4-13.3 g dry wt $m^{-2}d^{-1}$ by Goldman et al. (37). The production actually achieved in our flumes was 24 g dry wt $m^{-2}d^{-1}$, and our analysis of the production function indicates that about 26 g dry wt $m^{-2}d^{-1}$ could be achieved under optimal conditions. Thus we have been able to roughly double the best previous production figures from a P. tricornutum culture. This result is consistent with our earlier estimate (38), which indicated that use of foils in a flume culture system would increase production 2.2-2.4 fold.

The highest production that has been achieved on an extended basis in a culture growing solely on inorganic nutrients is 23 g dry wt $m^{-2}d^{-1}$ (34). That production figure was achieved with a freshwater culture of Scenedesmus over a 62-day period. On a short-term basis production as high as 35 g dry wt $m^{-2}d^{-1}$ has been achieved in cultures growing on wastewater or inorganic nutrients (34). My opinion is that one of the critical factors limiting production of P. tricornutum is the fact that the temperature of the culture must not rise much above 20°C. It is reasonable to assume that algae adapted to growth at higher temperatures would be able to fix carbon more rapidly at those higher temperatures. For example, Goldman et al. (37) report optimum production of about 13 g dry wt $m^{-2}d^{-1}$ in cultures grown at Woods Hole (latitude 41°52'N) and about 24 g dry wt $m^{-2}d^{-1}$ at Fort Pierce (latitude 27°28'N). The culture systems in both cases consisted of 2000 ℓ open-air ponds. The production results from both experiments pertain to results obtained during the May-October period. Although differences in irradiance undoubtedly account for some of the difference in the production figures, I suspect that part of the difference reflects the higher temperatures at the Fort Pierce site. It is noteworthy for example that at Woods Hole P. tricornutum dominated the cultures in May and June, and was subsequently replaced by Amphiphora sp. and later Amphora sp.. However, at Fort Pierce

Nitzschia closterium dominated the cultures over the entire study period. Evidently the ponds in the Fort Pierce study were too warm to allow P. tricornutum to become established. I suspect that production in our flumes can be significantly enhanced when we switch from P. tricornutum to some of the mesophilic species we are currently studying. The mesophilic species grow well in the temperature range 30-35°C, whereas P. tricornutum must be maintained at 20-25°C.

Ansell et al. (36) reported photosynthetic efficiencies averaging 3.7% over a two-week period in their P. tricornutum cultures. Under optimal conditions, our photosynthetic efficiencies averaged about 5.6% (Figure 3-6), about a 50% improvement over the best results achieved by Ansell et al. (36). The curves drawn in Figure 3-6 were obtained from an expansion similar to equation 4, and predict a maximum in photosynthetic efficiency at a pH of 7.5 and a dilution rate of 53% per day, values practically identical to those obtained from the analysis of the production data in Figure 3-5.

3.6 TASK III: ANALYSIS, MODELING, & LABORATORY SUPPORT STUDIES

- Objectives:
- To perform functional analysis to provide a common framework for the comparison of biological responses to physical treatments
 - To execute laboratory experiments which answer specific questions regarding algal response to culture conditions
 - To attempt to find strains of Phaeodactylum which are superior in terms of temperature tolerance and light utilization
- Purpose:
- To provide additional information on variables which cannot be adequately studied in the mass cultures

The specific subtasks performed under this task involved a) the development of a theoretical model to predict the potential increase in production which could be achieved utilizing the flashing light effect, b) the development of a cheap and effective means of harvesting the cells, c) an attempt to find strains of P. tricornutum better suited to mass culture than the strain we obtained from W. Thomas at Scripps, and d) the performance of various other laboratory experiments and analyses to elucidate physical, chemical, and biological processes in the mass culture and to test innovative culture manipulation strategies prior to their application in mass culture. We report the results obtained under each of these categories in the following discussion.

3.7 THEORETICAL ANALYSIS OF POTENTIAL ADVANTAGE OF FLASHING LIGHT EFFECT FOR MASS CULTURE WORK

This analysis is based on the experimental and theoretical studies

Figure 3-6. Photosynthetic efficiency in experimental flumes as a function of pH and dilution rate. Curved lines are expansion similar to equation 4.

of light-limited algal growth presented by Bannister (39). Under continuous light conditions we assume that algal growth can be described by the equations

$$\mu = (\mu_m + L') \xi / (\xi_g^n + \xi^n)^{1/n} - L' \quad (5)$$

$$= \left\{ \xi \phi_m K / [(\theta K)^m + \xi^m]^{1/m} - a \right\} / (1 + b) \quad (6)$$

$$\xi = K_c I \quad (7)$$

$$L' = a / (1 + b) \quad (8)$$

where μ is the growth rate (time^{-1}), I is the light irradiance ($\text{quanta} \cdot \text{area}^{-1} \cdot \text{time}^{-1}$), ξ is the specific absorbed irradiance ($\text{quanta absorbed} \cdot \text{mg chl } a^{-1} \cdot \text{time}^{-1}$), θ is the C:chl a ratio, and μ_m , K_c , ξ_g , ϕ_m , K , a , b , n and m are constants. The values assigned to these constants are listed in Table 3-7.

To apply the model to growth under light:dark cycle conditions, one assumes that the relationship between θ and μ under light-limited conditions is independent of photoperiod. Bannister (39) cites evidence to support this assumption. Although equation 5 applies only to growth under continuous light conditions, equation 6 can be modified to describe growth under light:dark cycle conditions by simply multiplying ϕ_m by f , the photoperiod expressed as a fraction of a day. For example, if the photoperiod is 16 hours, then $f = 2/3$. For our model we assumed a photoperiod of 12 h and therefore took $f = 0.5$.

Equation 5 can be inverted to solve for ξ as a function of μ . The result is

$$\xi = \xi_g (\mu + L') / [(\mu_m + L')^n - (\mu + L')^n]^{1/n} \quad (9)$$

The right-hand side of equation 9 can be substituted for ξ in equation 6. The resulting equation contains only two variables, μ and θ , and is the equation which determines the unique relationship between θ and μ . The resultant equation is

$$\theta = \left\{ \xi_g (\mu + L') / [(\mu_m + L')^n - (\mu + L')^n]^{1/n} \right\} \left\{ \phi_m^m / [\mu(1+b) + a]^{m-K^{-m}} \right\}^{1/m} \quad (10)$$

Now consider the case of growth under light:dark cycle conditions in a column of water in which the cells are slowly mixed. In that case it is reasonable to assume that the growth rate of the cells at each depth in the water column is described by equation 6, with ϕ_m replaced by $f\phi_m$ and with θ determined by equation 10. However, we know that θ cannot change on the same time scale (seconds or at most minutes) as the process of vertical mixing. It is therefore reasonable to assume that θ is the same for all the cells

Table 3-7. Parameter values used to characterize the Bannister (39) model.

μ_m (d^{-1})	2.5
K_c ($m^2 \cdot mg \text{ chl } a^{-1}$)	0.0105
ξ_g (Einst absorbed $\cdot mg \text{ chl } a \cdot d^{-1}$)	0.0462
ϕ_m ($gC \cdot Einst \text{ absorbed}^{-1}$)	0.7
K (Einst absorbed $\cdot gC^{-1} \cdot d^{-1}$)	5.7
a (d^{-1})	0.072
b (dimensionless)	0.117
n (dimensionless)	2.0
m (dimensionless)	3.0

in the culture. This value of θ is estimated by substituting $\bar{\mu}$, the average growth rate of all the cells in the culture, for μ in equation 10. We must therefore satisfy the following condition:

$$\bar{\mu} = \frac{1}{D(1+b)} \int_0^D \left\{ \xi f \phi_m K / [(\theta K)^m + \xi^m]^{1/m} - a \right\} dz \quad (11)$$

where θ is obtained from the right-hand side of equation 10 by substituting $\bar{\mu}$ for μ , and

$$\xi = \xi_0 e^{-K_c \cdot Z \cdot \text{chl } a} \quad (12)$$

In equation 12 ξ_0 is the specific absorbed irradiance at the surface of the culture, and we have assumed that all the attenuation of light in the culture is due to absorption by algal pigments. Obviously equation 11 is a transcendental equation in $\bar{\mu}$, but since we know that $0 \leq \bar{\mu} \leq \mu_m$, it is easy to solve the equation by the bisection method (40). In general the integration over depth can be handled numerically, and closed expressions are available for certain values of m .

Now the whole idea of utilizing the flashing light effect is to prevent the cells from responding to the light level at each depth, but instead to respond only to the average irradiance in the culture. In that case μ is obtained from the equation

$$\mu = \left\{ \bar{\xi} f \phi_m K / [(\theta K)^m + \bar{\xi}^m]^{1/m} - a \right\} / (1 + b) \quad (13)$$

where θ is obtained from equation 10 and $\bar{\xi}$ is the average specific absorbed irradiance over the depth of the culture. Hence

$$\bar{\xi} = \xi_0 (1 - e^{-K_c \cdot D \cdot \text{chl } a}) / (K_c \cdot D \cdot \text{chl } a) \quad (14)$$

Equation 13 is still a transcendental equation in μ , but again can be solved easily by the bisection method. Thus a comparison of the effect of flashing light on production in an algal culture can be made by comparing μ calculated from Equation 13 with $\bar{\mu}$ calculated from equation 11. The ratio $\mu/\bar{\mu}$ will depend both on the incident irradiance and on the areal chl a density ($\text{chl } a \cdot D$). In Hawaii the average irradiance in the visible region is about 100 Einst $\text{m}^{-2}\text{d}^{-1}$ (i.e. in a twelve hour photoperiod each m^2 receives 50 Einst). At production rates of 10 $\text{gC } \text{m}^{-2}\text{d}^{-1}$, our flume contains in the range of 0.5-1.0 $\text{g chl } a \text{ m}^{-2}$. Figure 3-7 shows the ratio $\mu/\bar{\mu}$ at three areal chl a densities and irradiances up to 100 Einst $\text{m}^{-2}\text{d}^{-1}$.

Several features about the plots are of interest. First, at very low incident irradiances there is no flashing light effect, because production is almost a linear function of ξ at low values of ξ . Second, at very low chl a densities there is no

Figure 3-7. Ratio of growth rate under ideal flashing light regime to growth rate in absence of flashing light effect as a function of incident irradiance and areal chl a concentration using equations 11 and 12.

flashing light effect, because $\xi \sim \bar{\xi}_0$. Third, at a chl a density of 0.5 g m^{-2} , there is a peak in the ratio $\mu/\bar{\mu}$ at $I_0 \sim 50 \text{ Einst m}^{-2}\text{d}^{-1}$. This peak occurs because as I_0 increases to high values $\bar{\xi}$ approaches an irradiance which almost saturates photosynthesis. In this case $\xi_0 \gg \bar{\xi}$, but if both ξ_0 and $\bar{\xi}$ are out on the asymptote of the μ versus ξ curve, the cells will respond to $\bar{\xi}$ in essentially the same way that they respond to ξ_0 . At chl $a = 1.0 \text{ g m}^{-2}$, the peak in the $\mu/\bar{\mu}$ ratio is evidently reached at $I_0 > 100 \text{ Einst m}^{-2}\text{d}^{-1}$. Finally, at chl a densities between 0.5 and 1.0 g m^{-2} , the model predicts that at $I_0 \sim 100 \text{ Einst m}^{-2}\text{d}^{-1}$, the flashing light effect can be expected to enhance production by somewhere between 80 and 225%. This theoretical prediction is consistent with our experimental results, which show enhancements between 50 and 100% in production and photosynthetic efficiency.

3.8 ALGAL HARVESTING STRATEGIES

We had hoped to be able to study the surface charge (Zeta potential) of the cells as a function of growth conditions, and to relate this surface charge to the tendency of the cells to flocculate and sink. Unfortunately we were not able to learn anything useful from these studies. The surface charge on the cells is apparently so low that there is virtually no movement in an electric field over the time period needed to perform the experiments. A technician on another project used the Zeta meter to study the surface charge on clay particles, and reported that the Zeta meter was working fine. Therefore the problem was not with the Zeta meter. All I can say at this point is that the Zeta potential on P. tricornutum cells is less than about 10 mV.

We were however successful in identifying a mechanism for harvesting the cells cheaply and efficiently. We had earlier explored the possibility of harvesting foam from the flume as a means of removing the cells in a concentrated form. However, because the optimum dilution rate is apparently 50-60% per day, we needed a harvesting mechanism which could deal with large volumes of water and remove the cells with virtually 100% efficiency. The foam harvester would be suitable if we wanted the water in the flume to have a very long residence time. However, that mode of operation would lead to very low production, probably because of the accumulation of waste products in the water.

I have earlier reported that simply raising the pH of the water through addition of NaOH causes a flocculation and rapid settling of the cells. We have since quantified this phenomenon. The cells flocculate rapidly when NaOH is added at a concentration of about 5mM. The sinking rate of the cells under these conditions is about three meters per hour. Let us assume for the

moment that we harvest half the volume of the flume each day, and that the flume is 10 cm deep. If we are willing to allow 12 hours for settling the cells, the surface area required for the settling operation would be $(0.1\text{m})(0.5)/[(3\text{m})(12)] = 0.14\%$ of the area of the flume itself. It is likely that much of the NaOH added to the water could be recovered as precipitated Na_2CO_3 along with the cells, and recycled many times.

3.9 SUITABILITY OF OTHER STRAINS OF PHAEODACTYLUM

We obtained five strains of P. tricornutum from the University of Texas culture collection, and examined the growth rate of these species as a function of temperature at 20, 25 and 28°C. The results are shown in Table 3.8. The principal discovery was that all of the University of Texas (UTEX) strains were able to grow at 28°C, whereas the Thomas strain is dead at 28°C. UTEX strains 640 and 642 showed substantially better growth at 28°C than did the other UTEX strains. The growth rates reported for the UTEX strains are in the cell concentration range $1-10 \times 10^6$ cells ml^{-1} ; the growth rates for the Thomas strain were obtained at cell concentrations of $10-30 \times 10^6$ cells ml^{-1} . Since growth rates decline as cell concentrations increase, the difference between the Thomas strain growth rates and the UTEX strains' growth rates at 20°C and 25°C are probably not significant. Since the temperature in our uncooled flumes can easily rise to 32-33°C during the day, it is doubtful if even the UTEX strains would be suitable for mass culture in Hawaii. UTEX strains 2089 and 2090 had lower lipid contents than the Thomas strain. The general conclusion is that there are strains of P. tricornutum more tolerant to high temperatures than the Thomas strain, but it is debatable whether these strains would do well in outdoor culture in Hawaii, and it also appears questionable whether lipid production would be increased by switching to one of the UTEX strains.

3.10 LABORATORY SUPPORT STUDIES

An experiment was conducted to determine the reproducibility of lipid analyses on samples taken from the experimental flumes. On eight consecutive days between 6/21/83 and 6/28/83 samples of the P. tricornutum culture were taken from experimental flume number four. The cells were analyzed for lipid content, and for the percentage of the lipid accounted for by neutral and polar lipids. The results are presented in Table 3-9. The results were highly reproducible.

During the course of the factorial experiment, we twice ran all four flumes under identical conditions to determine the reproducibility of the results. Table 3-10 summarizes the results of these two experiments. The results indicated that the

Table 3-8. Growth rate and lipid content of six strains of P. tricornutum.
Growth rates are in units of d^{-1} .

<u>Strain</u>	20°C	25°C	28°C
Thomas	0.31	0.14	—
UTEX 640	no data	0.20	0.23
UTEX 642	0.35	0.18	0.17
UTEX 646	0.45	0.20	0.11
UTEX 2089	0.42	0.10	0.12
UTEX 2090	0.44	0.20	0.13

Lipid Content
(% dry weight)

Thomas at 20.5°C	21% (16% neutral)
UTEX 2089 at 20°C	10% (23% neutral)
UTEX 2090 at 20°C	15% (23% neutral)

Table 3-9. Results of lipid analyses on flume P. tricornutum culture on eight consecutive days.

	<u>Mean</u>	<u>Standard Deviation</u>	<u>Range</u>
Lipids as % of dry weight	24.6	1.0	23.2 - 26.4
Neutral lipids as % of total lipids	22.5	3.0	18.4 - 26.4
Polar lipids as % of total lipids	77.5	3.0	73.6 - 81.6

Table 3-10. Summary of flume experiments to determine reproducibility of production results.

<u>Time Period</u>	<u>pH</u>	<u>Dilution Rate</u>	<u>Photosynthetic Efficiency</u>
7/8 - 7/19	8.3	30%	1.90 ± 0.40
8/25 - 8/28	7.7	50%	5.62 ± 0.39

standard deviation on the photosynthetic efficiency on a given flume was about 0.4%. We considered this result to be acceptable for our purposes.

At the January meeting in San Diego, Larry Raymond suggested to me that addition of selenium or nickel to the culture medium might result in an increase in the lipid content of P. tricornutum. To test this hypothesis cultures of P. tricornutum were grown in FCRG (Scripps Food Chain Research Group) medium, and exposed to concentrations of selenium and nickel from two to 1000 times normal seawater concentrations. Two 500 ml replicate batch cultures were inoculated at each of the three metal levels; two control cultures were grown in FCRG without additional Ni or Se. Nickel was added as nickel chloride; selenium was added as selenious acid. Cultures were also inoculated with two μ Curies of C-14 bicarbonate. Cultures were grown in continuous light and allowed to reach constant density ($3.5 - 4.0 \times 10^6$ cells ml^{-1}) before harvesting.

Subsamples from each culture were filtered onto glass fiber (GF/C) filters for triplicate particulate carbon and nitrogen analysis, and for triplicate analysis of the percent of the C-14 activity in the lipid fraction. The results are shown in Table 3-11. Data from replicate batch cultures have been combined, since there were no significant differences between replicate cultures. The percent of carbon allocated to lipids ranged from 29.3 to 32.4%. An analysis of variance showed no significant differences between treatments. Carbon:nitrogen ratios ranged between 5.9 and 6.6 by weight. Again there was no significant difference between treatments. Growth rates also appeared unchanged, since final cell concentrations were approximately equal after equal time periods. We concluded that adding selenium and/or nickel to the cultures was not worth pursuing further.

Table 3-11. Results of adding selenium and nickel to cultures of P. tricornutum.

<u>Treatment</u>	<u>Selenium added</u> <u>($\mu\text{g } \ell^{-1}$)</u>	<u>Nickel added</u> <u>($\mu\text{g } \ell^{-1}$)</u>	<u>% lipid</u>	<u>C:N (by weight)</u>
Control	0	0	32.4	6.63
Low metals	0.4	2.0	29.8	6.34
Medium metals	9	20	29.3	5.87
High metals	400	140	30.9	6.17

3.11 Task IV: PERFORMANCE SENSITIVITY ANALYSIS

Objective: • To prepare an evaluation of system inputs which quantifies operational parameters and identifies opportunities for improvement

Purpose: • To provide the data required to guide this emerging technology toward effective application

The following is an analysis of construction and annual maintenance costs of the experimental Algal Raceway Facility at the University of Hawaii Manoa. This section of the report will also compare the costs of a research facility to a production facility. After evaluating the data it became apparent that there are several areas in which price reductions could be significant. They are Personnel, Electricity, and Construction materials used in the raceway proper. i.e. Concrete versus Fiberglass flumes in a production system.

In evaluating the construction costs of the current experimental facility we analyzed the materials used in the four small flumes, which have a total area of 448 square feet. By adding up the entire construction material costs and dividing by the area we obtained a price per square foot. Basically the calculation went as follows:

Fiberglass	\$2,000.00
Plumbing	\$2,800.00
Foundation (raceway support system)	\$2,700.00
Cooling	\$6,800.00
Total	\$14,300.00
Divide by 448 sq. ft.	\$31.92/sq.ft.

Using this figure we were able to extrapolate the price in construction materials for moving from an experimental to a production system. The following four system options and their costs were calculated for an acre facility with 60% of the area under water (26,136 sq.ft.):

A) Fiberglass flumes with Cooling System	\$805,000.00
B) Concrete Flumes with Cooling	\$633,000.00
C) Concrete Flumes without Cooling	\$238,000.00
D) Fiberglass Flumes without Cooling	\$410,000.00

It should be noted that the concrete used in this estimate is a standard 4 inches thick; reduction of this thickness would decrease the cost considerably. Also this does not include land preparation.

Maintenance costs for the experimental facility were broken down into five categories

1) Office Expenses	\$6,100.00
2) Laboratory Expenses	\$8,300.00
3) Construction/System Maintenance	\$7,200.00
4) Electricity (with cooling)	\$19,200.00
(without)	\$6,000.00
5) Personnel	\$50,600.00
Totals with cooling	\$91,400.00
without cooling	\$78,200.00

The above categories include the following items: Office Expenses: mileage, photocopying, phone, and travel; Lab expenses: nutrients, chemicals, lab supplies and computer maintenance; Construction/System Maintenance: includes routine systematic maintenance and alterations of the system for experimental purposes; Electricity: includes routine facility power, and cooling costs for the raceways and culture rooms; Personnel: Includes 2 full time lab technicians at the university facility for analyzing results, 1 full time lab technician at the raceway for monitoring production, and 1 3/4 time maintenance person for system maintenance and experimental improvements.

In evaluating the transition from an experimental to production facility the following items were cited as possible areas for cost reduction:

Cooling: the cooling costs appear prohibitive in both the construction and maintenance areas

Personnel: the costs of personnel would be greatly reduced in a production facility due to a reduction in scientific analysis and research consideration. i.e. once there is a transition to production, questions on production variables will be settled.

REFERENCES

1. Burk, D., Cornfield, J., and Schwartz, M. 1951. The efficient transformation of light into chemical energy in photosynthesis. Sci. Monthly, 73: 213-223.
2. Clendenning, K.A. and Ehrmantraut, H.C. 1950. Photosynthesis and Hill reactions by whole Chlorella cells in continuous and flashing light. Arch. Biochem., 29: 387-403.
3. Emerson, R. and Arnold, W. 1932a. A separation of the reactions in photosynthesis by means of intermittent light. J. Gen. Physiol., 15: 391-420.
4. Emerson, R. and Arnold, W. 1932b. The photochemical reaction in photosynthesis. Jour. Gen. Physiol., 16: 191-205.
5. Rieke, F.F. and Gaffron, H. 1943. Flash saturation and reaction periods in photosynthesis. J. Phys. Chem., 47: 299-308.
6. Tamiya, H. and Chiba, Y. 1949. Analysis of photosynthetic mechanism by the method of intermittent illumination. Stud. Tokugawa Inst., 6: 1-129.
7. Weller, S. and Franck, J. 1941. Photosynthesis in flashing light. J. Phys. Chem., 45: 1359-1373.
8. Falkowski, P.G. and Wirick, C.D. 1981. A simulation model of the effects of vertical mixing on primary productivity. Mar. Biol., 65: 69-75.
9. Kok, B. 1953. Experiments on photosynthesis by Chlorella in flashing light, In: Algal Culture from Laboratory to Pilot Plant. Burlew, J.S. (ed). (Carnegie Inst. of Washington, Washington, DC), Publ. No. 600, pp. 63-75.
10. Kok, B. 1956. Photosynthesis in flashing light. Biochim. Biophys. Acta, 21: 245-258.
11. Markl, H. 1980. Modelling of algal production systems. In: Shelef, G. and Soeder, C.J. (eds). Algae Biomass. Elsevier-North Holland. Amsterdam. pp. 361-383.
12. Marra, J. 1978a. Effect of short-term variations in light intensity on photosynthesis of a marine phytoplankter: a laboratory simulation study. Mar. Biol. 46:191-201.
13. Marra, J. 1978b. Phytoplankton photosynthetic response to vertical movement in a mixed layer. Mar. Biol., 46: 203-208.
14. Phillips, J.N. and Myers, J. 1954. Growth rate of Chlorella in flashing light. Plant Physiol., 29: 152-161.

15. Powell, C.K., Chaddock, J.B., and Dixon, J.R. 1965. The motion of algae in turbulent flow. Biotech Bioeng. 7: 295-308.
16. Sager, J.C., and Giger, W. 1980. Re-evaluation of published data on the relative photosynthetic efficiency of intermittent and continuous light. Agricul. Meteor., 22: 289-302.
17. Fredrickson, A.G., and Tsuchiya, H.M. 1969. Utilization of the effects of intermittent illumination on photosynthetic microorganisms. In: Prediction and Measurement of Photosynthetic Productivity, Proceedings of the IBP/PP Technical Meeting, Trebon, September, 1969 (Centre for Agricultural Publications & Document, Wageningen, Netherlands). pp. 519-541.
18. Seibert, M., and Lavorel, J. 1982. Oxygen-evolution and patterns from oxygen-evolving photosystem II particles. In: Solar Energy Research Institute Biomass Program Principal Investigators' Review Meeting. Agenda and Abstracts. Washington, D.C., June 23-25, 1982. p. 17.
19. Miller, R.L., Fredrickson, A.G., Brown, A.H., and Tsuchiya, H.M. 1964. Hydromechanical method to increase efficiency of algal photosynthesis. Ind. Engng. Chem. Proces Des. Devel., 3: 134-143.
20. Davis, E.A., Dedrick, J., French, C.S., Milner, H.W., Myers, J., Smith, J.H.C., and Spoehr, H.A. 1953. Laboratory experiments on Chlorella culture at the Carnegie Institution of Washington department of plant biology. In: Algal Culture from Laboratory to Pilot Plant. Burlew, J.S., (ed). Carnegie Inst. of Washington, Washington, D.C. pp. 105-153.
21. Howell, J.A., Fredrickson, A.G., and Tsuchiya, H.M. 1966. Optimal and dynamic characteristics of a continuous photosynthetic algal gas exchanger. Chem. Engng. Prog. Symp. Ser. 62(68): 56-58.
22. Oswald, W.J., Golueke, C.G., and Horning, D.O. 1965. Closed ecological systems. Proc. Am. Soc. Civ. Engrs. (Sanitary Engineering), 91(SA4): 23-46.
23. Shelef, G., Sabanas, M., and Oswald, W.H. 1968. An improved algatron reactor for photosynthetic life support systems. Proc. 14th Ann. Techn. Meeting Inst. Envir. Sci.
24. Goldman, J.C., and Mann, R. 1980. Temperature-influenced variations in speciation and chemical composition of marine phytoplankton in outdoor mass cultures. J. Exp. Mar. Biol. Ecol., 46: 29-39.
25. Goldman, J.C., and Ryther, J.H. 1976. Temperature-influenced species competition in mass cultures of marine phytoplankton. Biotec. and Bioeng., 18: 1125-1144.
26. Ketchum, B.H., Lillick, L., and Redfield, A.C. 1949. The optimum growth and optimum yields of unicellular algae in mass culture. J. Cell. and Comp. Physiol., 33: 267-280.
27. Mann, J.E., and Myers, J. 1968. Photosynthetic enhancement in the diatom Phaeodactylum tricornutum. Plant Physiol., 43: 1991-1995.

28. McLachlan, J. 1964. Some considerations of the growth of marine algae in artificial media. Can. J. Microbiol., 10: 769-782.
29. Raymond, L.P. 1978. Initial Investigations of a shallow-layer algal production system. Hawaii Natural Energy Institute Tech. Rept. No. 7. 38 pp.
30. Goldman, J.C., and Stanley, H.I. 1974. Relative growth of different species of marine algae in wastewater-seawater mixtures. Mar. Biol., 28: 17-25.
31. Sharp, J.H. 1974. Improved analysis for "particulate" organic carbon and nitrogen from seawater. Limnol. Oceanogr., 19: 984-989.
32. Strickland, J.D.H. 1958. Solar radiation penetrating the ocean. A review of requirements, data and methods of measurement, with particular reference to photosynthetic productivity. J. Fish. Res. Bd. Canada, 15: 543-493.
33. Terry, K.L., Hirata, J., and Laws, E.A. 1983. Light-limited growth of two strains of the marine diatom Phaeodactylum tricornutum Bohlin: Chemical Composition, Carbon Partitioning and the Diel Periodicity of Physiological Processes. J. Exp. Mar. Biol. Ecol., 68: 209-227.
34. Goldman, J.C. 1979. Outdoor algal mass cultures - I. Applications. Water Res., 13: 1-19.
35. Aaronson, S., and Dubinsky, Z. 1982. Mass Production of microalgae. Experientia, 38: 36-40.
36. Ansell, A.D., Raymont, J.E.G., Lander, K.F., Crowley, E., and Shackley, P. 1963. Studies on the mass culture of Phaeodactylum. II. The growth of Phaeodactylum and other species in outdoor tanks. Limnol. Oceanogr., 8: 184-206.
37. Goldman, J.C., Ryther, J.H., and Williams, L.D. 1975. Mass production of marine algae in outdoor cultures. Nature, 254: 594-595.
38. Laws, E.A., Terry, K.L., Wickman, J., and Chalup, M.S. 1983. Results from a simple algal production system designed to utilize the flashing light effect. Biotech. Bioeng., in press.
39. Bannister, T.T. 1979. Quantitative description of steady state, nutrient-saturated algal growth, including adaptation. Limnol. Oceanogr., 24: 76-96.
40. Hornbeck, R.W. 1975. Numerical Methods. Quantum Publishers. New York. 310 pp.

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