SERI/STR-231-2496

UC Category: 61c

DE85000518

Research and Development of Shallow Algal Mass Culture Systems for the Production of Oils

A Subcontract Report

Edward A. Laws

**University of Hawaii
Honolulu, Hawaii**

October 1984

Prepared under Subcontract No. XK-3-03136

SERI Technical Monitor: Robins McIntosh

Solar Energy Research Institute

A Division of Midwest Research Institute

1617 Cole Boulevard
Golden, Colorado 80401

Prepared for the

U.S. Department of Energy

Contract No. DE-AC02-83CH10093

Printed in the United States of America
Available from:
National Technical Information Service
U.S. Department of Commerce
5285 Port Royal Road
Springfield, VA 22161
Price:
Microfiche A01
Printed Copy A04

NOTICE

This report was prepared as an account of work sponsored by the United States Government. Neither the United States nor the United States Department of Energy, nor any of their employees, nor any of their contractors, subcontractors, or their employees, makes any warranty, express or implied, or assumes any legal liability or responsibility for the accuracy, completeness or usefulness of any information, apparatus, product or process disclosed, or represents that its use would not infringe privately owned rights.

The 48-m² outdoor raceway system showing installed foils

FOREWORD

This report is the final report for FY 1984. 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 to investigate the mass culture of microalgae as a source of renewable fuels.

Robins P. McIntosh
Aquatic Species Program

Michael Z. Lowenstein
Biomass Program Office

Approved for the

SOLAR ENERGY RESEARCH INSTITUTE

Stanley R. Bull, Director
Solar Fuels Research Division

SUMMARY

Objective

The objective of this project was to determine whether microalgae could be grown successfully in a shallow outdoor flume without temperature control, to determine the yields which could be achieved from such a system, and to evaluate the merits of such mass culture for the purposes of energy production from an economic and energetic standpoint.

Discussion

A shallow flume system for the mass culture of microalgae 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. Airlift pumps, restricted within 15 cm PVC pipes, are used for recirculation and gas exchange. 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, are suspended in the flowstream at a relatively high angle of attack. Vortices are generated at the tips of each of these foils and are propagated downstream. Arrays of foils are positioned every 1.2m along the culture flume. Observation of the vortex rotation rates have indicated that cells are exposed to a light-dark cycle time of 1 to 2 seconds in this mixing regime. This light-dark cycling avoids prolonged exposure of the cells to bright light and associated low photosynthetic efficiencies. Productivity in a system employing the foils is about 2.2 times the productivity in the same system without foils.

A major accomplishment of the past nine months' work was the identification of a microalgal species which can be grown in the system on a 12-month basis without temperature control. The most promising species identified to date is a strain of Platymonas sp.. This strain grows rapidly at temperatures from 20 to 34°C, and at salinities from 15 to 35 ‰. Neither the lower temperature limit nor the lower salinity limit of the strain are known at this time. A factorial experiment designed to determine optimum growth conditions indicated that the optimum culture depth was 10 cm, the optimum pH about 7.5, and the optimum flow rate about 30 cm/sec. A major discovery was that diluting the culture every third day greatly enhanced production. In this dilution mode daily yields averaged 46 g/m² ash-free dry weight (AFDW) over a one-month period, and photosynthetic efficiencies averaged 11% (based on visible light energy). The former figure is over twice the best long-term yields achieved in microalgal mass culture systems grown exclusively on inorganic nutrients.

Conclusions

An economic analysis of the shallow outdoor flume system indicated that if photosynthetic efficiencies of 11% can be maintained, then energy from algal biomass will be cost competitive with crude oil at a crude oil price of \$57 per barrel. This calculation assumes that the microalgae are grown without the benefit of temperature controls, a clearly achievable goal if Platymonas or a similar eurythermal species is the cultured organism. Use of temperature controls to grow a less thermophilic species such as Phaeodactylum tricornutum would more than double production costs. An energetic analysis of the flume system indicated that the major energy inputs were the energy required to provide fixed nitrogen, the energy required to operate the airlift pumps, and the energy required for harvesting and processing. However, if the entire energy content of the microalgae is utilized, the energy output of the system is over four times the energy input.

TABLE OF CONTENTS

	<u>Page</u>
1.0 Introduction	
1.1 Historical Background	1
1.2 Theoretical Background	2
2.0 Materials and Methods	
2.1 The Culture System	4
2.2 Analytical Data	13
3.0 Results	
3.1 Task I: Screening of Candidate Species in Laboratory Cultures	13
3.2 Task II: Yield Optimization of Promising Species in Shallow Outdoor Flumes	14
3.3 Fractional Factorial Experiments with Platymonas	17
3.4 Nutrient Starvation Studies with Platymonas	23
3.5 Long Term Production Run with Platymonas	23
3.6 Efforts to Grow Other Species	35
3.7 Task III: Analysis of Promising Culture Management Strategies	36
4.0 References	41
5.0 Appendix	44

LIST OF FIGURES

	<u>Page</u>
1-1 Design of a single foil indicating mechanism of vortex production	5
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. Arrows indicate rotational direction of vortices	6
2-1 Perspective of algal production flume showing location of lift box, drain box, and airlift system	7
2-2 Cross section of flume modules indicating positioning of airlift and CO ₂ feed	9
2-3 Detail of recirculation and airlift system	10
2-4 Schematic of seawater supply system and algal storage tank	12
3-1 Growth of four species of marine microalgae at 34°C in IMR medium	16
3-2 <u>Platymonas</u> production as a function of pH and dilution rate. Curved lines are fit of Eq. 1 to data	18
3-3 C/N ratios during period of nutrient starvation in <u>Platymonas</u> culture	24
3-4 Lipid content of <u>Platymonas</u> cultures during nutrient starvation study	25
3-5 Long term <u>Platymonas</u> production and photosynthetic efficiency results in 48m ² flume. Data points are six-day running means	27

LIST OF TABLES

	<u>Page</u>
2-1 Flume specifications.	8
3-1 Log phase growth rates (doublings per day) of four strains of microalgae which grew at temperatures of 32°C and 34°C.	15
3-2 Production results in 9.2m ² flumes operated under identical conditions. Culture depth was 10 cm and dilution rate 50% per day in all cases. Production values are daily means (gC m ⁻²) over a one-week period.	20
3-3 Production results in 9.2m ² flumes run at a depth of 10 cm and dilution rate of 50% per day. pH was controlled at 7.5 either by CO ₂ bubbling or addition of HCl. Results are average daily production rates (gC m ⁻²) over the indicated number of days.	21
3-4 First-order factorial production results in 9.2m ² flumes with <u>Platymonas</u> . Culture depth was 10 cm, dilution rate 50% per day. Results are daily production rates (gC m ⁻²) averaged over a two-week period.	22
3-5 Compositional analysis of <u>Platymonas</u> cells at start of nutrient starvation experiment (in parentheses) and after 14 days of starvation.	26
3-6 Monthly means of solar radiation (PAR) and maximum and minimum water temperatures in 48m ² flume during January - May, 1984.	28
3-7 Mean particulate organic carbon and ash-free dry weight concentrations in the 48m ² flume during three-day dilution cycle experiments. Units are g m ⁻³ .	32
3-8 Summary of lipid analyses on <u>Platymonas</u> culture from 9.2m ² flume during three-day dilution cycle. Lipid content expressed as % of AFDW.	33
3-9 Production results from 9.2m ² flumes operated at a pH of 7.5 and depth of 10 cm during April-May, 1984.	34
3-10 Estimated costs associated with operation of a shallow algal mass culture facility expressed on a per acre-year basis.	37

Estimates based on operation of a 1000 acre facility (33).

3-11	Materials and energy inputs required to produce <u>Platymonas</u> in shallow outdoor flume.	38
3-12	Annual energy balance for shallow algal mass culture system expressed on a per acre water surface area basis. Estimates based on operation of a 1000 acre facility (33).	40

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 48m² continuous flume; the development of predator control techniques and baseline performance characteristics for the flume; and the construction of four 9.2m² experimental flumes.

A major contribution of the project during these early years was the development of a system that introduced 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. Productivity in the system employing the foils was increased by a factor of 2.2 over the productivity in the same system without foils.

During the 1982-83 funding period the four 9.2m² experimental flumes were operated in a factorial design experiment to determine the maximum sustainable biomass yields achievable with P. tricornutum cultured in shallow flume systems. Dilution rate and CO₂ supply were found to be the most important system parameters. Maximum production was found to be about 25 g ash-free dry wt $m^{-2}d^{-1}$. This production corresponded to a photosynthetic efficiency of 5.6%. These figures were 50-100%

better than the production rates achieved in earlier P. tricornutum cultures using conventional culture techniques. The results were found to be consistent with a theoretical model of the impact of the flashing light effect on algal mass culture production. This model predicted 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 created systematic vertical mixing on a time scale suitable for utilizing the flashing light effect. It was found that harvesting P. tricornutum cells could be accomplished straightforwardly by adding approximately 0.5 mM NaOH to the culture. With this addition the cells rapidly flocculated and settled out with almost 100% efficiency..

Although high yields were achieved with P. tricornutum during this period of study, a serious problem with the use of P. tricornutum in outdoor algal cultures became apparent. P. tricornutum grows poorly if at all when temperatures exceed 25°C. In parts of the U.S. best suited for algal mass culture growth, water temperatures during the summer months, the prime production season, will routinely exceed 25°C. If the water were cooled in order to permit growth of P. tricornutum, the energy inputs to the system would exceed the energy produced. It therefore became obvious that strains of microalgae capable of growing in the temperature range 25-35°C would have to be identified, and their production capability in shallow outdoor mass culture systems determined. The primary goal of the past year's research was therefore to determine maximum sustainable biomass yields achievable with thermophilic species of marine microalgae cultured in outdoor shallow flume systems. Secondary goals 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 effect on production of growing thermophilic strains during warm weather and P. tricornutum during cooler weather as a means of minimizing biomass production costs. It was expected that these goals would be achieved through work on the following three tasks:

- I Screening of candidate species in laboratory cultures
- II Yield optimization of promising species in outdoor flumes
- III Analysis of promising culture management strategies

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 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 algatron, 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 0.5-1.0 mm 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 1.2 x 2.4m 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 modules are reinforced with split 7.5 cm diameter polyvinyl chloride (PVC) pipe, and are mounted on 5 x 10 cm wooden rails supported by concrete blocks.

In addition to the channel modules, there are two end-box modules and 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

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

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. Arrows indicate rotational direction of vortices

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

TABLE 2-1.

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	48.39 m 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	
Construction	2-cm plywood painted with fiberglass resin
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
Area	2-cm plywood painted with fiberglass resin 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 2-2. Cross section of flume modules indicating positioning of airlift and CO₂ feed.

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

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 Fuller Co. 7.5 horsepower model 4MV-F air blower. CO₂ gas additions are made utilizing an aquarium airstone at a depth of 1m in the downward-flowing portion of the recirculation pipes.

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 μ M ammonium and 0.8 μ M 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 seawater pump is controlled on the basis of the operator input and the output of the level detector. Carbon dioxide gas (100% pure) addition is controlled on the basis of pH. Draining is controlled on a time-of-day basis.

Laboratory culture experiments were performed using IMR medium (24) sterile-filtered through 0.22 μ m Millipore membrane filters. Polycarbonate culture tubes (25mm x 150mm) were placed in a temperature controlled ($\pm$ 0.1°C) water bath in front of a bank of daylight fluorescent bulbs which supplied light at an irradiance of 450 μ Einst m⁻²s⁻¹. Light was provided on a 12:12 L:D illumination cycle. The biomass of the cultures in each tube was monitored through measurements of in vivo fluorescence on a Turner model 10 fluorometer. Fluorescence measurements were made at the same time each day to avoid artifacts associated with light:dark periodicity.

The four 9.2m² flumes are similar in design to the 48m² flume, but consist of two channels 7.6m long by 0.6m wide by 0.15m deep. The channels are connected by 20 cm recirculation pipes; one of the two recirculation pipes of each culture contains an airlift

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

pump which drives culture recirculation. Each 9.2m² flume is equipped with a CO₂ supply line. pH sensors in the cultures supply data to the Hewlett-Packard 9845B computer, which in turn controls the CO₂ supply solenoids.

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 (25). These carbon production numbers were converted to ash-free dry weight production by multiplying by the experimentally reported ratio of ash-free dry weight:PC. For Platymonas (= Tetraselmis) this ratio is 2.1 (26). The proximate composition of the algae was obtained by partitioning the ash-free dry weight (AFDW) between lipid, protein and carbohydrate. The lipid content was estimated using a modified Bligh-Dyer extraction. The protein content was estimated to be 6.25 times the nitrogen content (26). Carbohydrate content was estimated by difference, i.e. carbohydrate = AFDW - lipid - protein.

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 (i.e. photosynthetically active radiation or PAR). Visible quantum fluxes were converted to energy fluxes using the spectral distribution of sunlight (27). The conversion ratio was 19.5 μ Einst per calorie. The caloric content of the microalgae was calculated from the proximate analysis and energy contents of 9.3 kcal g⁻¹ for lipid, 4.2 kcal g⁻¹ for carbohydrate, and 5.7 kcal g⁻¹ for protein (28). 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: SCREENING OF CANDIDATE SPECIES IN LABORATORY CULTURES

- Objective: o To determine which microalgal species are most likely to grow well at elevated temperatures in culture systems, and to select three or more for outdoor yield studies
- Purpose o To reduce the number of species which must be studied in the outdoor flume factorial experiments

Most marine phytoplankton do not grow well at temperatures in excess of 30°C. For example, of seven tropical diatom isolates from Surinam, three exhibited no growth at 33°C, and the remaining four exhibited no growth at 37°C (29). However, some species exhibit remarkable thermal tolerance. A strain of Chaetoceros gracilis has been reported to grow optimally in the temperature range 23-37°C, and to be capable of growth in the temperature range 11-41°C (30). Since the temperature in our flumes typically reaches 34-35°C for an hour or two during each day in the summer, we felt that the ability of the cultured organism to grow at temperatures approaching 35°C would be necessary if the flumes were to be operated without temperature controls.

Table 3-1 and Figure 3-1 show the results from our laboratory studies for those species which we found capable of growth at 32°C and 34°C. None of these species exhibited any growth at 36°C. The best overall growth at 32 and 34°C was displayed by Platymonas sp., a species which initially appeared as a contaminant in our outdoor flumes during studies of P. tricornutum. Our experience with this species both in the laboratory and in the outdoor culture system indicates that it grows well over the temperature range 20-34°C, and is therefore a promising candidate for year-round culture in Hawaii or in the southwestern U.S. A culture of Platymonas sp. was sent to Dr. William Barclay at SERI for inclusion in the SERI culture collection. Thalassionema nitzschoides and Chaetoceros gracilis closely followed Platymonas with respect to growth rate at 32 and 34°C. The culture of the former species was subsequently lost as a result of an electrical problem with our incubator. However, both C. gracilis and Isochrysis survived, and were subsequently tested for production potential in the experimental outdoor flumes. It is noteworthy that C. gracilis did not grow at 36°C, although the strain is reportedly identical to the strain which was earlier reported to grow at 11-41°C (30). Our results indicate that this strain may have lost some of its thermal tolerance after almost 20 years in culture collections.

3.2 TASK II: YIELD OPTIMIZATION OF PROMISING SPECIES IN SHALLOW OUTDOOR FLUMES

- Objectives:
- o To determine how much algal production can be expected from shallow outdoor flumes without temperature control
 - o To determine the sensitivity of the production function to changes in design characteristics
- Purpose
- o To determine whether the production of shallow outdoor flumes can be maintained or even enhanced by foregoing temperature control and by growing thermophilic microalgae during the warm summer months

Table 3-1. Log phase growth rates (doublings per day) of four strains of microalgae which grew at temperatures of 32°C and 34°C.

	Source	Temperature	
		32°C	34°C
<u>Chaetoceros gracilis</u>	Univ. British Columbia culture collection	0.66	0.69
<u>Isochrysis</u> (Tahitian)	R. York, Hawaii Inst. Mar. Biol. culture collection	0.45	0.71
<u>Platymonas</u> sp.	isolated from our mass culture flume	0.65	0.82
<u>Thalassionema nitzschioides</u>	Kaneohe Bay, Hawaii	0.56	0.83

Figure 3-1. Growth of four species of marine microalgae at 34°C in IMR medium

3.3 FRACTIONAL FACTORIAL EXPERIMENTS WITH PLATYMONAS

The species most thoroughly studied in the outdoor flumes was Platymonas. During our earlier work with P. tricornutum (31) we performed a first-order factorial experiment in which the following parameters were each assigned two values:

1. culture depth
2. dilution rate
3. water temperature
4. flow rate
5. presence of CuSO_4 filters
6. pH at which CO_2 additions begin
7. NH_4^+ or urea as N source
8. salinity

In the present studies no effort was made to control water temperature, and CuSO_4 filters were not used. The decision to eliminate the CuSO_4 filters was based on the fact that the filters themselves remove 50% of incoming visible light, and although our work with P. tricornutum indicated that photosynthesis was more efficient in blue light, the increase in efficiency was consistently less than the factor of two necessary to make up for the loss of 50% of the visible light. First order factorial experiments with Platymonas indicated that

1. production was as good at 15 ‰ as at 35 ‰ salinity
2. NH_4^+ and urea were equally good sources of N (not a surprising result, since $\text{CO}(\text{NH}_2)_2 + \text{H}_2\text{O} \rightarrow 2\text{NH}_3 + \text{CO}_2$)
3. production is higher at a flow rate of 30 cm/s than at 15 cm/s
4. production is insensitive to culture depth between 8 and 12 cm, but is reduced at shallower and deeper depths
5. production is a sensitive function of dilution rate and the pH at which CO_2 addition begins

Since a flow rate of 30 cm/s was close to the maximum we could achieve with our airlift system, we chose to conduct subsequent experiments at that flow rate. Salinity was set at 35 ‰, culture depth at 10 cm, and NH_4^+ was chosen as the N source. A second-order factorial design experiment in which dilution rate and pH were varied was then used to determine the optimum production conditions. The results are shown in Figure 3-2. Each data point represents mean results obtained over at least a one-week period. Note that dilution rates in this figure refer to once-a-day dilutions, not to continuous dilution rates. For example, our 50% dilution rate (requiring a doubling per day to maintain steady state cell numbers) would correspond to a continuous dilution rate of 69.3% per day.

In order to describe the experimental results, an analytical function of the form

$$Y = C_0 + C_1 \mu + C_2 \text{pH} + C_3 \mu^2 + C_4 \text{pH}^2 + C_5 \mu \text{pH} + C_6 \mu^4 \quad (1)$$

Figure 3-2. Platynonas production as a function of pH and dilution rate. Curved lines are fit of Eq. 1 to data

was fit by least squares to the data. In Equation 1 Y is the response function (production or photosynthetic efficiency), μ is the dilution rate, and pH is the pH at which CO_2 additions began. The optimum production obtained from the fit to the data in Figure 3-2 was $10.9 \text{ gC m}^{-2}\text{d}^{-1}$ at a pH of 7.3 and a dilution rate of 53%. The corresponding photosynthetic efficiency based on PAR was 7%. Since $pH = 7.0$ corresponds to having the CO_2 bubbling essentially all the time during the photoperiod, there was no reason to explore lower pH's. Furthermore, setting the pH at 7.0 results in a very low efficiency of CO_2 utilization ($\sim 25\%$; see later discussion). Since production is rather insensitive to pH in the pH range 7.0 - 7.5, we elected to conduct further experiments in the large flume at a pH of 7.5.

Two experiments were run to check on the reproducibility of results, and the results are shown in Table 3-2. The results indicate that the standard deviations are 5-10% of the means. As a further check we repeated certain conditions in individual flumes on different weeks, and obtained very similar results. For example at $pH = 7$ and $\mu = 40\%$, production on two successive weeks was 10.29 and 10.64 $\text{gC m}^{-2}\text{d}^{-1}$, and at $pH = 7.5$ and $\mu = 50\%$, production on two successive weeks was 11.32 and 11.68 $\text{gC m}^{-2}\text{d}^{-1}$. Thus results were reasonably reproducible from week to week and from flume to flume.

The principal accomplishment of this phase of the research was to show that good production could be achieved in a shallow outdoor flume system without temperature control. In fact, the results obtained from the factorial studies with Platymonas were slightly better than those obtained using a temperature-controlled system with P. tricornutum (31). Removal of the temperature control constraint was a big step towards making the culture operation energetically favorable.

Tables 3-3 and 3-4 contain the results of some exploratory experiments we performed in the small flumes with Platymonas before setting up a long-term production run in the large flume. The results in Table 3-3 were obtained at a dilution rate of 50% per day and a pH of 7.5. However, in two cases we controlled pH by bubbling CO_2 , and in the other two cases by adding HCl. Production was obviously much better when CO_2 was used to control pH. The conclusion from this experiment is that CO_2 additions are necessary to obtain high production rates, and that the influx of CO_2 from the atmosphere is not adequate to keep up with the demands of the algae, even if the pH is maintained at 7.5 with acid so as to prevent the conversion of dissolved CO_2 to bicarbonate at higher pH's. The data in Table 3-4 were in fact obtained as part of our first-order factorial studies, and indicate that production is not significantly affected by salinity in the range 15-30‰ or by culture depth between 8 and 12 cm. In all cases the dilution rate was 50% per day and the flow rate 30 cm/s.

Table 3-2. Production results in 9.2 m² flumes operated under identical conditions. Culture depth was 10 cm and dilution rate 50% per day in all cases. Production values are daily means (g C m⁻²) over a one-week period.

flume	pH = 8	pH = 7.5
1	11.2	11.3
2	9.1	11.4
3	10.8	12.5
4	9.6	11.5
mean ± SD	10.2 ± 1.0	11.7 ± 0.6

Table 3-3. Production results in 9.2 m² flumes run at a depth of 10 cm and dilution rate of 50% per day. pH was controlled at 7.5 either by CO₂ bubbling or addition of HCl. Results are average daily production rates (gC m⁻²) over the indicated number of days.

flume	pH control	Production	duration of run (days)
1	HCl	8.6 ± 3.2	6
2	HCl	9.2 ± 4.5	6
3	CO ₂	13.2 ± 3.5	4
4	CO ₂	11.8 ± 3.0	6

Table 3-4. First-order factorial production results in 9.2 m² flumes with Platymonas. Culture depth was 10 cm, dilution rate 50% per day. Results are daily production rates (gC m⁻²) averaged over a two-week period.

culture depth (cm)	Salinity (‰)	
	15	30
8	9.0 ± 3.1	9.4 ± 1.9
12	9.3 ± 2.1	10.6 ± 3.5

3.4 NUTRIENT STARVATION STUDIES WITH PLATYMONAS

Figures 3-3 and 3-4 illustrate the results of a nutrient starvation study with Platymonas performed in the small flumes during November, 1983. At the start of the experiment all four flumes were in the same condition, and had been operated in a nutrient-saturated mode at a dilution rate of 50% per day and pH = 7.5 for a period of one week. Beginning on day one P additions were terminated to one flume, N additions were terminated to a second flume, and both N and P additions were terminated to a third flume. One flume continued to be operated in a nutrient saturated mode. As can be seen in Figure 3-3, the C/N ratios in the nutrient-saturated and P-limited flumes showed no significant temporal variation over the subsequent two-week period, and there was no significant temporal trend in the lipid content of these flumes over a time period of almost three weeks (Figure 3-4). Thus P limitation had no significant impact on the lipid content or gross composition of the cells. However, the C/N ratio in the N-limited cells increased from about 6.5 to 8 by weight during the second week of starvation (Figure 3-3), and the C/N ratio of the N and P limited cells steadily increased from about 6.5 to over 12 by weight between days 4 and 14 of the starvation. Thus there was evidence that both N limitation and N + P limitation triggered an increase in the percentage of carbon storage products in the cells, with N + P limitation producing the most rapid and much greater accumulation of storage products. Figure 3-4 however shows that the percentage of lipids in the N limited and N + P limited cells actually declined steadily from day 5 to day 19 of the starvation. The measured C/N ratios and lipid composition values combined with the fact that the ratio of AFDW to carbon in Platymonas is 2.1 can be used to perform a crude compositional analysis of these cells, and the results are shown in Table 3-5. It is clear from the results in this table that the increased C/N ratios observed in the N-limited and N + P limited cultures reflect carbohydrate storage rather than lipid storage, and that the increased carbohydrate content is balanced largely by a reduction in the protein content. We conclude from these studies that Platymonas can be induced to produce storage products by means of nutrient starvation, that simultaneous N + P limitation is the most effective way to induce the production of these storage products, that the storage products will be carbohydrates, and that the increase in the carbohydrate content of the cells will be balanced largely by a reduction in their protein content.

3.5 LONG TERM PRODUCTION RUN WITH PLATYMONAS

On 1/1/84 we began a five-month continuous run with Platymonas in the 48m² flume. The results of this run in terms of AFDW production and photosynthetic efficiency based on PAR are shown in Figure 3-5. Table 3.6 lists monthly means for solar radiation (PAR) and maximum and minimum water temperatures during the study period. Daily data have been tabulated in the appendix. The purpose of this experiment was to operate the large flume in the

Figure 3-3. C/N ratios during period of nutrient starvation in Platymonas culture.

Figure 3-4. Lipid content of *Platymonas* cultures during nutrient starvation study.

Table 3-5. Compositional analysis of Platymonas cells at start of nutrient starvation experiment (in parentheses) and after 14 days of starvation.

	% protein	% lipid	% carbohydrate
	(47)	(18)	(35)
nutrient-saturated	46	18	36
P-limited	46	18	36
N-limited	36	15	49
N + P-limited	24	15	61

Figure 3-5. Long term *Platymonas* production and photosynthetic efficiency results in 48m^2 flume. Data points are six-day running means

Table 3-6. Monthly means of solar radiation (PAR) and maximum and minimum water temperatures in 48m² flume during January - May, 1984.

<u>Month</u>	Solar Radiation (PAR, Einst m ⁻² d ⁻¹)	Water Temperature (°C)	
		<u>Minimum</u>	<u>Maximum</u>
January	35.3	21.6 ± 1.1	30.6 ± 2.0
February	36.7	20.9 ± 1.4	30.2 ± 1.4
March	45.7	21.4 ± 1.1	32.2 ± 1.1
April	49.5	21.7 ± 0.8	31.8 ± 1.7
May	54.0	22.4 ± 0.7	32.8 ± 1.7

mode which the earlier factorial experiments had indicated should be optimal, and to determine what sort of long term production results could be obtained. During the early portion of this run such a policy was followed, but other events and discoveries led to modifications in our operating procedures which ultimately proved highly beneficial. During the first few months of the study we attempted to operate both the 48m² flume and the four small flumes. Two problems became apparent during this time. First, the amount of air generated by our 3.5 horsepower air compressor was inadequate to produce proper flow rates in all the flumes. This problem was corrected by installation of a 7.5 horsepower air blower in mid-February. The second problem concerned the quality of the water we obtained from our saltwater well. This water is anoxic when it comes out of the ground, and pretreatment by means of air bubbling is necessary before the water can be used in the flumes. Unfortunately our well water storage tank (Figure 2-4) was not large enough to treat the water needed to run all five flumes for a sufficient period of time (two days or more). Thus some compromises had to be struck in the dilution schedules until changes could be made in our plumbing system. The changes in the system consisted of installing valves which allowed the well water to be pumped into the algal storage tank (Figure 2-4), which has a much larger capacity than the well water storage tank. With this modification it was possible to give the well water adequate pretreatment before using it in the flumes.

The result of this latter problem is that the large flume was diluted on a somewhat irregular basis until 2/27/84. In some cases the flume was diluted every day for several days; in other cases periods of several days went by when the flume was diluted every other day. Daily production during this time averaged 22.8 g AFDW m⁻², and the average photosynthetic efficiency was 7.4%. The irregular dilution pattern turned out to be a blessing in disguise. By scrutinizing the data, we discovered that production tended to be higher on the second day after dilution than on the first day. We therefore hypothesized that diluting the culture every other day might result in higher yields than once daily dilutions. We tested this hypothesis by diluting the large flume every two days for a period of 28 days from 2/27/84 to 3/25/84. During the first 14 days the culture concentration was reduced to 2×10^6 cells ml⁻¹ after dilution, and during the second two weeks the culture concentration after dilution was 3×10^6 cells ml⁻¹. Production was slightly better when the concentration was reduced to 3×10^6 cells ml⁻¹, but the principal result of the study was that production was consistently higher on the second day after dilution. In other words, growth following each dilution was not a linear function of time, but instead gave evidence of being exponential. The average production on the first day after dilution was 23.3 g AFDW m⁻², and the average daily production on the second day after dilution was 35.6 g AFDW m⁻². Overall daily production during this one-month time period averaged 29.6 g AFDW m⁻².

Given these results, we hypothesized that diluting the culture

every three days might result in even higher production. We therefore set up a 30-day run from 3/26/84 to 4/26/84 during which time the culture was diluted every three days. The cell concentration after dilution was 2×10^6 cells ml^{-1} . A heavy rain on 4/20/84 effectively diluted the culture on two successive days (4/19 and 4/20), and rainy and cloudy conditions during the last week of the study period resulted in relatively low production. However, the average daily production over the 30-day run was $45.9 \text{ g AFDW m}^{-2}$. The most remarkable aspect of the results was the consistently high daily production achieved on the third day after dilution, almost 78 g AFDW m^{-2} . However, daily productions on the first and second days following dilution were not significantly different, and averaged 33 ± 10 and $27 \pm 9 \text{ g AFDW m}^{-2}$. The average photosynthetic efficiency over the 30-day run was 11.0%.

Because of the excellent production results achieved with the three-day dilution cycle, we hypothesized that a four-day dilution cycle might be even better, and tested this hypothesis by conducting a 20-day experiment from 4/26/84 to 5/16/84 in which the 48m^2 flume was diluted every four days. However, daily production during this time averaged only $27.2 \text{ g AFDW m}^{-2}$. Daily production on the four days after dilution averaged 26, 36, 33 and 14 g AFDW m^{-2} . There was obviously no day on which production was consistently high, and production on the fourth day was much below that on the other three days. We concluded that a four-day dilution cycle was clearly inferior to a three-day dilution cycle.

From 5/16/84 to 6/2/84 we diluted the 48m^2 flume every third day in order to check the reproducibility of the previously observed production pattern, and to perform some additional analyses to try to understand why production was so high when the cells were grown in this mode. The daily production during this time period averaged $40.0 \text{ g AFDW m}^{-2}$, and daily production on the three days following each dilution averaged 34.3, 21.7, and $64.0 \text{ g AFDW m}^{-2}$, respectively. The three-day production pattern was thus qualitatively similar to the pattern observed from 3/26 to 4/26 in that production on the third day was 2-3 times greater than the average production on the first two days, and production on the second day was lower than on the first day.

Photosynthetic efficiencies averaged 11.0% and 8.3% during the 3-day dilution cycle runs from 3/26 to 4/26 and from 5/16 to 6/2, respectively. However, photosynthetic efficiencies on the third day after dilution averaged 18.8% from 3/26 to 4/26. Since the maximum possible photosynthetic efficiency is believed to be about 20% (32), we hypothesized that some of the particulate carbon production observed on the third day after dilution might represent nonphotosynthetic production. We postulated that the cells might have been releasing dissolved organic carbon (DOC) into the water during the first two days after dilution, and then reassimilating the excreted DOC on the third day. To test this hypothesis we measured DOC concentrations in one of the 9.2m^2 flumes between 5/7/84 and 5/13/84, during which time the flume

was being operated in a three-day dilution mode. The DOC concentrations ranged between 4.5 and 11.5 g C m⁻³. At the same time, particulate organic carbon (POC) concentrations varied from 101 g C m⁻³ immediately after dilution to 687 g C m⁻³ three days later. The average increase in POC during the third day after dilution was 399 g C m⁻³. Clearly such increases in POC could not be accounted for by uptake of DOC if the DOC values never exceeded 11.5 gC m⁻³. We concluded that the high production observed on the third day after dilution must indeed result from photosynthesis on that day. Table 3-7 summarizes the temporal trend in POC concentrations in the 48m² flume during the three-day dilution cycle experiments. The data are averages from the periods 3/26 to 4/19 and 5/16 to 6/2. The week of heavy rains from 4/20 to 4/26 has been excluded.

Table 3-8 summarizes lipid analyses on Platymonas grown in one of the 9.2 m² flumes from 5/7/84 to 5/12/84 on a three-day dilution cycle. There was no dramatic change in the lipid content of the algae over the course of the dilution cycle, nor was there much change in the relative amount of neutral and polar lipids. Thus the very high rate of production on the third day after dilution was not associated with any dramatic change in the lipid content of the cells.

Table 3-9 summarizes the results of a small-scale factorial experiment in the 9.2 m² flumes during April and May to reexamine the relative merit of three and four day dilution cycles and the effect of cell concentration on production. The best results were again obtained with the three-day dilution cycle, and diluting the cell concentration to 2 x 10⁶ cells ml⁻¹ was preferable to diluting to 4 x 10⁶ cells ml⁻¹. During this time a careful check was made on the demand for CO₂ by the cultures. The combined four flumes consumed one 50 lb. tank of 99.8% CO₂ in an average time of 7.5 days. Since the average rate of production over this time interval was 0.47 (37.9 + 33.8 + 29.5 + 29.4)/4 = 15.3 gC m⁻²d⁻¹, the efficiency of CO₂ utilization was (9.2)(4)(15.3)(7.5)/[(50)(454)(12/44)] = 69%.

Our outdoor flume results with Platymonas can be summarized as follows. By diluting the culture every three days to a concentration of about 2 x 10⁶ cells ml⁻¹ (100 gC m⁻³), photosynthetic efficiencies of about 11% can be achieved. These high efficiencies are clearly associated with the establishment of a production rhythm, in which peak production and efficiencies (19%) occur on the third day after dilution. The average insolation in Hawaii is about 5000 kcal m⁻²d⁻¹, of which about 50% is in the visible portion of the spectrum (P. Ekern, pers. comm.). Given the Platymonas energy content of 12.3 kcal g⁻¹C and the fact that carbon accounts for 47% of the AFDW of Platymonas, Platymonas production could be expected to average (5000)(0.5)(0.11)/[(12.3)(0.47)] = 47.6 g AFDW m⁻²d⁻¹ over the course of a year. Platymonas showed excellent growth throughout the year in our outdoor flumes, and we conclude that this species can grow rapidly at temperatures from approximately 20°C to 34°C. The lower temperature limit of the species is not known at this

Table 3-7. Mean particulate organic carbon and ash-free dry weight concentrations in the 48m² flume during three-day dilution cycle experiments. Units are g m⁻³.

<u>Days Since Dilution</u>	POC	AFDW
0	100 ± 19	213 ± 40
1	224 ± 24	477 ± 51
2	316 ± 20	672 ± 43
3	612 ± 123	1,302 ± 262

Table 3-8. Summary of lipid analyses on Platymonas culture from 9.2 m² flume during three-day dilution cycle. Lipid content expressed as % of AFDW.

Days after dilution	Lipid content	% neutral lipids	% polar lipids
1	15.6 ± 0.5	25.2 ± 0.8	74.8 ± 0.8
2	15.0 ± 0.6	29.4 ± 0.6	70.6 ± 0.6
3	17.5 ± 0.1	26.5 ± 0.9	73.5 ± 0.9

Table 3-9. Production results from 9.2 m² flumes operated at a pH of 7.5 and depth of 10 cm during April-May, 1984.

<u>Dilution Interval (days)</u>	<u>Cell conc. just after dilution (millions/ml)</u>	<u>Duration of run (days)</u>	<u>Daily Production (g AFDW m⁻²)</u>
3	2	18	37.9
3	4	18	33.8
4	2	16	29.5
4	4	16	29.4

time. Over the 20-34°C temperature range Platymonas appears to have no significant competitors, and during our study we saw no evidence of predation. Platymonas is capable of excellent growth in salinities as low as 15 ‰; the lower salinity limit of the species is not known at this time. The lipid content of Platymonas equals 15-18% of the AFDW; N starvation or N+P starvation leads to the accumulation of carbohydrate.

3.6 EFFORTS TO GROW OTHER SPECIES

After we had completed the factorial studies with Platymonas in the 9.2m² flumes, we attempted to carry out similar studies with three other species. Tahitian Isochrysis studies were begun in February. Although we were able to establish dense cultures initially, the flumes were rapidly taken over by Platymonas. Efforts to establish and maintain Isochrysis cultures continued for several weeks into March, but the cultures were invariably taken over by Platymonas. In April we attempted to begin a factorial study with a saltwater strain of Chlorella which laboratory studies had indicated would grow at 30-34°C. The result was very similar to the Isochrysis results. Dense initial cultures were established, but the flumes were again taken over by Platymonas. We were simply unable to maintain anything resembling unialgal outdoor cultures of either Isochrysis or Chlorella for a sufficient period of time to carry out a meaningful factorial study before the cultures become dominated by Platymonas. It is noteworthy here that Platymonas grows well in a medium containing a minimum of nutrient additions (N, P, and trace metals), whereas both Isochrysis and Chlorella required vitamin additions to achieve satisfactory growth. Thus it is impossible to discriminate against Platymonas and in favor of some other species by leaving out a particular nutrient, unless of course one is trying to grow a nitrogen-fixing species, in which case N may obviously be omitted.

In late April we set up a culture of Chaetoceros gracilis in a 3m² flume which we had originally constructed for hydrodynamic studies with foil arrays. The flume was not designed in an optimal way for microalgae growth, but production appeared to be good, and we began to take samples for particulate carbon analysis on April 30. Over the subsequent three-week period production averaged 35.5 g AFDW m⁻²d⁻¹, and there was no evidence of Platymonas contamination. Encouraged by these results, we began a factorial experiment in the 9.2m² flumes with C. gracilis in late May. Unfortunately the flumes were again gradually taken over by Platymonas. Since C. gracilis requires additions of both silicate and vitamins, there again is no chance to discriminate against Platymonas on the basis of nutrient additions.

In summary, we have been unable to find a species which can effectively compete with Platymonas in an outdoor flume containing a nutrient-enriched seawater medium. This picture may obviously change as we move into studies involving southwestern U.S. water types during the next phase of research. Discrimination against Platymonas on the basis of nutrient

additions to seawater is impossible, since Platymonas itself requires an absolute minimum of nutrient additions. The only exception to this generalization is the N requirement, where growth of N-fixing blue-green algae would obviously be possible without N additions.

3.7 TASK III: ANALYSIS OF PROMISING CULTURE MANAGEMENT STRATEGIES

Objective: o To prepare an evaluation of system inputs which quantifies operational parameters and determines the costs and benefits of operating the shallow outdoor flumes with and without temperature controls, in the latter case by substituting thermophilic microalgae for P. tricornutum during the warm months of the year

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

Our experience with Platymonas has indicated that this species can be grown throughout the year in Hawaii. Certainly Platymonas can be grown successfully at temperatures low enough to permit culture of P. tricornutum without temperature control. Thus it is clear that a mass culture system utilizing either Platymonas exclusively or Platymonas and P. tricornutum could be successfully operated on a year-round basis either in Hawaii or in the southwestern U.S.

The cost of operating a flume mass culture facility similar in design to the system we have been using is estimated in Table 3-10. The estimated cost of the facility is \$12,401 per acre-year. If it were necessary to cool the flumes in order to grow P. tricornutum throughout the year, the additional cost would be about \$13,200 per acre-year (31). Table 3-11 gives a complete breakdown of the materials and energy inputs required to produce Platymonas.

It is worthwhile at this point to consider the value of the product. If production averages $47.6 \text{ g AFDW m}^{-2}\text{d}^{-1}$ (i.e. 11% photosynthetic efficiency in Hawaii), then annual production would be 77.5 tons AFDW per acre-year. If the flumes cover about 80% of the surface area of the production facility, then the break-even value of the product would have to be $(\$12,401)/[(77.5)(2000)(0.8)] = \0.10 per pound. If we assume the energy content of a barrel of crude oil to be $6 \times 10^6 \text{ BTU}$ ($= 1.5 \times 10^6 \text{ Kcal}$) (33), then the microalgal biomass produced in our facility would be cost competitive with crude oil at a crude oil price of $(\$0.10)(1.5 \times 10^6)/[(0.454)(0.47)(12.3 \times 10^3)] = \57 per barrel. However, if it were necessary to cool the flumes in order to grow P. tricornutum throughout the year, the cost of an equivalent barrel of oil would have to be $(\$57)(\$12,401 + \$13,200)/(\$12,401) = \$118$ per barrel. We conclude that cooling the flumes would more than double the cost of the product, and would make the product uncompetitive with crude oil for the

Table 3-10. Estimated costs associated with operation of a shallow algal mass culture facility expressed on a per acre-year basis. Estimates based on operation of a 1000 acre facility (33).

I.	Total Capital Investment	\$32,450
II.	Direct Costs - nutrients, water, direct labor, maintenance, power, harvesting, and processing	\$4,569
III.	Indirect Costs - taxes, insurance, depreciation, land lease	\$1,645
IV.	General and Administrative - administration, interest, return on investment	<u>\$6,187</u>
	Total Operating Costs per Acre-year	\$12,401

Table 3-11. Materials and energy inputs required to produce Platymonas in shallow outdoor flume. Calculations assume that water is recycled after harvesting cells. Thus nitrogen and phosphorus requirements pertain to nutrients removed with the cells, and water requirements pertain to evaporation losses. Evaporation losses assumed to be 1 cm per day.

<u>Requirement</u>	<u>Quantity/kg AFDW</u>
CO ₂	2.5 kg
Nitrogen	83 g
Phosphorus	11 g
Water pumping and mixing	173 kcal
Water	210 liters

likely foreseeable future. However, if supplemental cooling is eliminated, the cost of an equivalent barrel of crude oil is \$57, a figure which may well be reached within the next 20 years.

Perhaps the most obvious energy input to the system is the power needed to circulate water through the flumes. The difference in the elevation of water at the high and low point of our 48m² flume is 5 cm. The flow rate is 30 cm/sec, the water depth is 10 cm, and the width of the flume is 130 cm. Therefore in one second the airlifts must move $(130)(10)(30) = 39,000$ cc of water a distance of 5 cm. The power input is therefore $(39,000)(5) \text{ gm-cm/sec} = 4.57 \text{ cal/sec} = 395 \text{ Kcal/day}$. If production averages $47.6 \text{ g AFDW m}^{-2}\text{d}^{-1}$, the energy output is $(47.6)(0.47)(12.3)(48) = 1.32 \times 10^4 \text{ Kcal/day}$. Hence the energy needed to move the water is $(395)(100)/(1.32 \times 10^4) = 3.0\%$ of the energy output.

Table 3-12 provides a more complete energy balance for the facility (33). The principal energy input is actually the energy required for the nutrients, in particular the energy required for the production of fixed nitrogen. The energy required to operate the airlifts and the energy required for harvesting and processing make up most of the remaining energy inputs. Overall the energy inputs amount to a little less than 24% of the energy produced in the form of biomass. Thus the energy balance for the facility is quite favorable, at least as long as supplemental cooling is not used to control water temperatures.

In summary, we conclude that the major cost of operating a temperature-controlled shallow flume algal mass culture facility would be the cost of cooling the water. This cost would account for a little over 50% of the expense of producing the algae. However, if the temperature control constraint is eliminated (e.g. if a species such as Platymonas is grown), the biomass produced by the facility would be cost-competitive with crude oil at a crude oil cost of \$57 per barrel. The major energy inputs to the system would be the energetic cost of producing fixed nitrogen, the energy required to operate the airlifts, and the energy required for harvesting and processing. However, the energy output is estimated to be over four times the energy input. It is noteworthy here that these calculations assume that all the energy content of the product algae is utilized. If only the energy contained in the lipid portion of the cells were utilized, neither the economics nor the energy balance of the project would be favorable.

Table 3-12. Annual energy balance for shallow algal mass culture system expressed on a per acre water surface area basis. Estimates based on operation of a 1000 acre facility (33).

	<u>Energy Inputs (10^6 Kcal)</u>
Materials	
General	2.6
Nutrients	68.6
Pumping	
Make-up water	0.3
Airlifts	11.8
Delivery	0.7
Piping	0.07
Harvest/Processing	8.9
Total Energy Input	93.0
Energy of Biomass Produced	393

4.0 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. Eppley, R.W., R.W. Holmes and J.D.H. Strickland. 1967. Sinking rates of marine phytoplankton measured with a

- fluorometer. J. Exp. Mar. Biol. Ecol. 1: 191-208.
25. Sharp, J.H. 1974. Improved analysis for "particulate" organic carbon and nitrogen from seawater. Limnol. Oceanogr., 19: 984-989.
 26. Parsons, T.R., K. Stephens and J.D.H. Strickland. 1961. On the chemical composition of eleven species of marine phytoplankters. J. Fish. Res. Bd. Canada 18: 1001-1016.
 27. 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.
 28. Milner, H. W. 1953. The Chemical Composition of Algae. In: Algal culture: from laboratory to pilot plant. Burlew, J. S. (ed). Carnegie Inst. of Washington, Washington, D.C. chapter 19.
 29. Hulbert, E. M. 1982. In: New trends in research and utilization of solar energy through biological systems. Mislin, H., and Bachofen, R. (eds).
 30. Thomas, W. H. 1966. Effects of temperature and illuminance on cell division rates of three species of tropical oceanic phytoplankton. J. Phycol. 2: 17-22.
 31. Laws, E.A. 1984. Research, development, and demonstration of algal production raceway (APR) systems for the production of hydrocarbon resources. Solar Energy Research Institute subcontract No. XE-0-9013-01 report.
 32. Goldman, J. C. 1979. Outdoor algal mass cultures - II. Photosynthetic yield limitations. Water Res. 13: 119-136.
 33. Raymond, L.P. 1978. Initial investigations of a shallow-layer algal production system. Hawaii Natural Energy Institute Tech. Rept. No. 7. 38 pp.

APPENDIX

Daily production, AFDW, cell counts, irradiance and photosynthetic efficiencies in 48m² flume during January - May, 1984. Photosynthetic efficiencies based on PAR. Double entries on a given day for culture density and cell counts are values before and after dilution on that day.

DAILY PRODUCTION, ASH-FREE DRY WEIGHTS, CELL COUNTS AND PHOTOSYNTHETIC EFFICIENCY

DATE	LIGHT INTENSITY (E/m ² d)	CULTURE DENSITY (g/l AFDW)	CELL COUNT (10 ⁶ ml)	PRODUCTION AFDW (g/m ² d)	EFFICIENCY (%)
1 JAN 84	35.907	.38	4.8	11.915	3.741
2 JAN 84	35.907	.46	4.1	12.128	3.807
3 JAN 84	35.907	.55	6.3	18.511	5.811
4 JAN 84	35.907	.68/.36	6.8/3.3	13.617	4.275
5 JAN 84	35.907	.45/.23	4.5/3.0	14.468	4.542
6 JAN 84	24.882	.34/.18	3.3/2.3	19.787	8.965
7 JAN 84	37.108	.51/.17	5.7/3.4	28.298	8.597
8 JAN 84	37.108	.40	5.9	18.936	5.753
9 JAN 84	37.108	.55/.31	5.7/3.7	18.723	5.688
10 JAN 84	37.108	.46	4.5	22.979	6.981
11 JAN 84	41.184	.65/.30	8.5/3.8	16.596	4.543
12 JAN 84	28.958	.44	5.1	25.957	10.105
13 JAN 84	39.065	.65/.26	6.9/2.3	15.957	4.538
14 JAN 84	29.065	.39/.14	6.0/2.3	18.936	7.344
15 JAN 84	29.065	.28	4.0	12.766	4.951
16 JAN 84	29.065	.37/.19	5.6/2.3	11.489	4.456
17 JAN 84	29.065	.27	3.9	26.170	10.150
18 JAN 84	31.788	.47/.15	7.1/1.9	18.085	6.414
19 JAN 84	28.185	.28	4.4	38.936	15.573
20 JAN 84	40.154	.58/.13	6.9/1.8	21.489	6.033
21 JAN 84	30.952	.29	4.7	17.660	6.432
22 JAN 84	30.952	.43/.11	7.5/1.6	25.319	9.221
23 JAN 84	35.467	.30	3.7	18.511	5.883
24 JAN 84	35.467	.44/.14	6.5/1.6	25.319	8.048
25 JAN 84	39.983	.33	4.2	27.021	7.618
26 JAN 84	41.656	.54/.13	6.7/1.5	30.638	8.291
27 JAN 84	41.827	.15	---	31.702	8.544
28 JAN 84	39.683	.40	6.4	22.128	6.286
29 JAN 84	39.683	.57/.36	8.2/4.1	19.362	5.500
30 JAN 84	39.511	.51/.47	9.1/4.7	8.723	2.489
31 JAN 84	39.511	.53/.21	10.6/2.5	13.404	3.824
1 FEB 84	34.792	.29	5.2	23.830	7.721
2 FEB 84	39.124	.45/.20	7.4/1.7	8.723	2.514
3 FEB 84	30.931	.26	3.1	27.234	9.926
4 FEB 84	34.250	.43	3.1	29.574	9.734
5 FEB 84	34.250	.62/.27	5.8/2.0	18.085	5.952
6 FEB 84	33.938	.38	2.0	13.191	4.382
7 FEB 84	33.938	.47/.20	6.3/1.8	21.915	7.279
8 FEB 84	30.974	.34	3.4	26.170	9.525
9 FEB 84	38.738	.51/.22	5.3/1.7	24.894	7.244
10 FEB 84	36.072	.37	3.9	43.404	13.564
11 FEB 84	33.497	.65/.22	6.6/1.8	28.936	9.738
12 FEB 84	31.789	.40	4.6	19.574	6.941
13 FEB 84	31.789	.53/.90	5.9/1.5	25.319	8.979
14 FEB 84	34.811	.29	3.8	31.064	10.059
15 FEB 84	30.581	.49/.23	5.0/1.9	17.660	6.510

16 FEB 84	35.148	.37	3.9	34.043	10.918
17 FEB 84	31.578	.64	6.4/2.1	51.489	18.381
18 FEB 84	36.128	.87/.43	5.5	36.915	11.518
19 FEB 84	36.128	.72/.25	8.6/2.0	22.340	6.971
20 FEB 84	39.714	.42/.16	5.1	18.830	5.345
21 FEB 84	39.714	.30	7.9/3.5	15.319	4.348
22 FEB 84	39.714	.43	5.3	37.021	10.509
23 FEB 84	38.621	.72/.25	8.7/2.1	23.617	6.893
24 FEB 84	43.844	.44	4.9	27.660	7.112
25 FEB 84	41.227	.65/.26	10.8/3.2	22.340	6.109
26 FEB 84	41.227	.44	6.4	19.362	5.294
27 FEB 84	45.619	.59/.24	9.4/3.1	19.362	4.784
28 FEB 84	42.557	.39	5.6	49.787	13.188
29 FEB 84	43.271	.78/.25	9.0/2.5	25.106	6.541
1 MAR 84	39.873	.45	7.1	39.574	11.189
2 MAR 84	38.511	.75/.18	8.1/2.3	21.277	6.228
3 MAR 84	38.643	.34	4.9	16.170	4.717
4 MAR 84	35.276	.47/.16	6.7/1.8	20.851	6.663
5 MAR 84	43.544	.32	2.8	33.830	8.758
6 MAR 84	43.544	.58/.20	7.4/2.1	32.766	8.483
7 MAR 84	42.731	.46	5.0	48.511	12.798
8 MAR 84	42.731	.84/.27	10.5/3.3	25.745	6.792
9 MAR 84	43.869	.47	7.7	45.319	11.646
10 MAR 84	43.581	.82/.24	11.6/3.0	27.660	7.155
11 MAR 84	42.691	.45	6.5	27.660	7.304
12 MAR 84	48.714	.60/.37	7.0/3.3	18.298	4.234
13 MAR 84	50.278	.52	7.1	50.851	11.401
14 MAR 84	53.024	.91/.28	12.2/3.4	24.255	5.157
15 MAR 84	44.745	.47	7.1	32.128	8.094
16 MAR 84	44.988	.72/.14	8.5/3.0	26.170	6.558
17 MAR 84	44.988	.34	7.8	26.170	6.558
18 MAR 84	44.988	.55/.28	6.6/3.2	20.213	5.065
19 MAR 84	54.269	.44	5.9	20.851	4.331
20 MAR 84	58.001	.60/.29	7.2/3.1	16.170	3.143
21 MAR 84	53.003	.41	5.0	10.851	2.308
22 MAR 84	53.003	.50/.27	4.9/2.2	20.426	4.344
23 MAR 84	55.427	.43	5.1	20.000	4.068
24 MAR 84	44.951	.59/.24	6.7/2.7	27.872	6.990
25 MAR 84	50.284	.46	5.2	76.170	17.076
26 MAR 84	48.771	1.05/.18	9.1/1.8	29.787	6.885
27 MAR 84	48.263	.41	4.4	27.234	6.361
28 MAR 84	39.468	.62	7.3	91.915	26.253
29 MAR 84	40.562	1.34/.22	11.7/1.6	35.319	9.816
30 MAR 84	41.656	.49	5.6	28.085	7.600
31 MAR 84	41.656	.71	13.7	93.191	25.219
1 APR 84	47.276	1.43/.23	15.2/1.7	34.043	8.117
2 APR 84	53.496	.49	5.0	30.000	6.322
3 APR 84	49.507	.73	9.9	123.617	28.148
4 APR 84	51.094	1.69/.20	17.1/2.1	36.809	8.121
5 APR 84	46.160	.49	6.9	27.021	6.599
6 APR 84	44.058	.70	8.3	90.000	23.028
7 APR 84	48.434	1.40/2.0	15.8/1.7	27.021	6.289
8 APR 84	48.477	.42	7.0	28.723	6.679
9 APR 84	43.496	.64	7.0	96.170	24.925
10 APR 84	48.434	1.39/.14	14.9/1.4	44.255	10.300

11 APR 84	51.437	.49	6.4	20.000	4.383
12 APR 84	53.325	.64	9.9	104.468	22.085
13 APR 84	50.432	1.50/.16	13.4/1.3	32.128	7.181
14 APR 84	51.394	.41	5.2	34.681	7.607
15 APR 84	50.429	.68	9.8	26.170	5.850
16 APR 84	50.997	.88/.29	10.7/2.6	28.936	6.396
17 APR 84	50.997	.51	5.9	27.021	5.973
18 APR 84	51.565	.72	9.0	120.638	26.373
19 APR 84	56.872	1.60/.16	15.5/1.6	14.468	2.868
20 APR 84	42.128	.27/.12	2.8/1.0	56.809	15.201
21 APR 84	42.084	.56	4.2	18.936	5.072
22 APR 84	58.763	.70	10.8	23.404	4.490
23 APR 84	48.263	.89/.25	10.0/2.5	31.277	7.305
24 APR 84	58.004	.49	6.7	11.277	2.190
25 APR 84	48.391	.58	7.2	7.872	1.834
26 APR 84	31.489	.64/.18	8.8/1.6	23.830	8.531
27 APR 84	48.734	.36	4.1	44.894	10.385
28 APR 84	50.836	.72	-----	44.894	9.955
29 APR 84	50.836	1.06	14.3	-6.596	-1.463
30 APR 84	58.044	1.01/.29	14.5/2.4	17.021	3.306
1 MAY 84	56.971	.42	5.8	28.936	5.726
2 MAY 84	39.983	.64	8.7	35.319	9.958
3 MAY 84	49.817	.92	13.8	35.532	8.040
4 MAY 84	48.734	1.19/.21	15.6/1.8	34.681	8.022
5 MAY 84	59.116	.48	6.8	42.766	8.155
6 MAY 84	44.402	.82	10.9	18.936	4.808
7 MAY 84	66.409	.96	12.4	23.404	3.973
8 MAY 84	55.212	1.15/.23	-----	24.255	4.952
9 MAY 84	45.559	.42	-----	35.319	8.739
10 MAY 84	58.044	.70	8.9	35.957	6.983
11 MAY 84	61.046	.98	10.9	-17.021	-3.143
12 MAY 84	29.515	.85/.29	9.3/2.0	31.064	11.865
13 MAY 84	60.746	.53	5.6	26.170	4.857
14 MAY 84	65.208	.73	5.8	28.298	4.892
15 MAY 84	55.770	.95	10.4	62.766	12.687
16 MAY 84	52.810	1.44/.19	17.5/2.5	32.553	6.949
17 MAY 84	57.958	.44	5.9	25.106	4.883
18 MAY 84	56.843	.64	7.9	29.787	5.907
19 MAY 84	58.148	.87/.20	13.3/2.3	27.234	5.280
20 MAY 84	58.149	.42	4.2	22.128	4.290
21 MAY 84	59.454	.59	4.9	55.957	10.610
22 MAY 84	49.506	1.03/.22	10.9/2.3	34.681	7.897
23 MAY 84	64.135	.49	5.6	19.574	3.441
24 MAY 84	52.424	.64	8.1	52.340	11.255
25 MAY 84	38.438	1.05/.23	12.9/2.6	33.617	9.859
26 MAY 84	55.598	.50	5.7	26.170	5.306
27 MAY 84	53.539	.70	8.4	67.234	14.157
28 MAY 84	59.145	1.22/.24	14.5/2.8	39.574	7.543
29 MAY 84	57.443	.55	7.2	20.000	3.925
30 MAY 84	49.206	.70	10.7	102.128	23.397
31 MAY 84	53.797	1.50/.27	14.8/2.4	37.660	7.891

Document Control Page	1. SERI Report No. SERI/STR-231-2496	2. NTIS Accession No.	3. Recipient's Accession No.
4. Title and Subtitle Research and Development of Shallow Algal Mass Culture Systems for the Production of Oils		5. Publication Date October 1984	
7. Author(s) Edward A. Laws		6.	
9. Performing Organization Name and Address University of Hawaii Honolulu, Hawaii		8. Performing Organization Rept. No.	
		10. Project/Task/Work Unit No. 4625.10	
		11. Contract (C) or Grant (G) No. (C) XK-3-03136-1 (G)	
12. Sponsoring Organization Name and Address Solar Energy Research Institute 1617 Cole Boulevard Golden, Colorado 80401		13. Type of Report & Period Covered Technical Subcontract Report	
		14.	
15. Supplementary Notes Technical Monitor: R. McIntosh			
16. Abstract (Limit: 200 words) The major accomplishment of the past nine months' work was the identification of a microalgal species which can be grown in the system on a 12-month basis without temperature control. The most promising species identified to date is a strain of <u>Platymonas sp.</u> This strain grows rapidly at temperatures from 20° to 34°C, and at salinities from 15 to 35‰. Neither the lower temperature limit nor the lower salinity limit of the strain are known at this time. A factorial experiment designed to determine optimum growth conditions indicated that the optimum culture depth was 10 cm, the optimum pH about 7.5, and the optimum flow rate about 30 cm/s. A major discovery was that diluting the culture every third day greatly enhanced production. In this dilution mode daily yields averaged 46 g/m ² ash-free dry weight (AFDW) over a one-month period, and photosynthetic efficiencies averaged 11% (based on visible light energy). The former figure is over twice the best long-term yields achieved in microalgal mass culture systems grown exclusively on inorganic nutrients.			
17. Document Analysis a. Descriptors Algae; Chlorella; Cost; Efficiency; Productivity; Seawater; Unicellular Algae b. Identifiers/Open-Ended Terms Chaetoceros Gracilis; Isochrysis; Platymonas c. UC Categories 61c			
18. Availability Statement National Technical Information Service U.S. Department of Commerce 5285 Port Royal Road Springfield, Virginia 22161		19. No. of Pages 57	
		20. Price A04	