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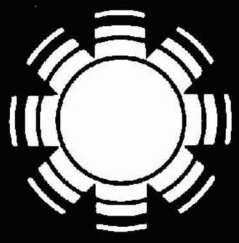
A Final Subcontract Report

J. C. Weissman
R. P. Goebel

Microbial Products, Inc.
Fairfield, California

Prepared under Subcontract No. XK-3-03136



SERI

Solar Energy Research Institute
A Division of Midwest Research Institute

1617 Cole Boulevard
Golden, Colorado 80401

Operated for the
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FOREWORD

This is the final report on work completed under FY 1983 and FY 1984 funding. 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

Approved for

SOLAR ENERGY RESEARCH INSTITUTE

Stanley R. Bull, Acting Director
Solar Fuels Research Division

SUMMARY

OBJECTIVES

An overall objective of the project was to conceptually determine if simple open pond systems have application for the production of fuels from microalgae. To demonstrate the overall objective, work concentrated on showing the potential microalgal yields that are possible from an open pond system on a sustained basis. Furthermore, problems (pond management, design) associated with this experimental system were documented and reported so that future endeavors shall benefit. Finally, operational costs were documented to permit preliminary economic analysis of the system. Specific objectives that were set for fulfillment of this contract were:

- demonstrate the outdoor monoculture potential of at least four species of promising oleagenous microalgae.
- demonstrate sustained yields (4 mo.) of at least 2 species of promising oleagenous microalgae.
- identify specific management strategies that are applicable to the open pond system.
- identify cost centers, and report operational expenses associated with the present operations.
- conduct experimental work aimed at reducing the operational costs associated with the system.

Under this contract, the species selected to be grown was deemed very important. A primary objective of this work was to determine the suitability and productivities of microalgae designated as "promising" by other subcontractors working on this project. Thus species selection for outdoor work was determined with inputs and accord of other subcontractors and the SERI program coordinator. Once species were identified that grew well outdoors, work was initiated so as to establish management strategies for maximization of productivities. Specifically, this work compared batch vs. continuous culture and mixing speeds. Cost centers were identified and operational costs recorded and reported for subsequent economic analyses and comparisons.

The specific tasks set for this project were:

TASK I. Inoculate and maintain in outdoor ponds at least 2 species of microalgae that have been identified as "promising" by ASP researchers involved in screening and development.

TASK II. Compare the productivities from two pond management strategies; batch and continuous culture.

TASK III. Acclimation of Isochrisis galbana from a salinity of seawater (35 ppt) to a slightly brackish salinity of 5 ppt.

TASK IV. Determine the cost-productivity trade-offs of chemicals (chelators, trace metals) presently added to the medium.

TASK V. Growth of microalgae on recycled media.

TASK VI. Maintain two species of the previously selected microalgae in the outdoor ponds for a period of at least four months in a production mode that will best demonstrate the potential of the system.

DISCUSSION

All of the tasks outlined for this project were addressed during project performance. Of the strains of microalgae provided by other ASP subcontractors, none could outcompete invading wildtype algae. Nonetheless, two strains, Ankistrodesmus falcatus and Scenedesmus So2a were grown outdoors in mass culture, one for two months, the other for one month. Results obtained using these strains did provide information useful to the screening program in the future. Two wildtype organisms, Scenedesmus quadricauda and Chlorella sp. provided most of the outdoor results. The Scenedesmus was maintained for thirteen months, averaging about 13 $gm/m^2/day$. It was used to compare batch vs. continuous cultivation, for mixing speed experiments, for media recycling, for storage product induction tests, and for the determination of operational costs. The Chlorella was grown for two months at over 20 $gm/m^2/day$, and was used to determine the relationship between mixing velocity and productivity.

The maintenance of a monoculture was not difficult when climatic conditions were relatively constant. Changes of species, from Scenedesmus to Chlorella, occurred when pond temperatures rose above 35°C and reversed when the temperatures dropped. Both wildtypes tolerated a range of TDS and mineral composition. Ankistrodesmus tolerated most medium composition variations, except low K^+, but was sensitive to high irradiance when in dilute suspension. None of the organisms required chelator, trace supplementation (beyond that available in the water used), or vitamin supplementation. The results indicate that a monoculture can be maintained if either the seasonal temperature variations are small or a competitive strain is used which has a broad temperature optimum. A competitive strain is one which does not exhibit significant photoinhibition, oxygen inhibition or respiratory losses. Of course, TDS will exert an overall selection criterion.

As mentioned above, the productivity results obtained with the "summer" Chlorella and the Scenedesmus during the remainder of the year, indicate that a year round average of 15-20 $gm/m^2/day$ is attainable in this northern California climate with the strains used. A yearly average of over 20-25 $gm/m^2/day$ could probably be obtained 500 miles south, where ambient

temperatures are higher during spring, fall and winter. Continuous cultivation increased production relative to sequential batch operation, by 30% during summer. However, the typical density of the culture at harvest time was much lower under continuous cultivation. In addition, induction of storage products required one to three days of batch growth after a continuous or batch active growth stage.

Mixing velocity experiments revealed four factors of importance in considering optimum mixing speed: CO_2 outgassing which increases quickly with mixing speed, power input which increases even more quickly, suspension of cells, and productivity response. It was found that 15-20 cm/sec mixing speed was sufficient to keep even the largest (1-2mm) clumps of Scenedesmus suspended. It was also found that, from 1 to 60 cm/sec, mixing speed had no effect on productivity.

Experimental results indicated that organisms respond very differently to pH and CO_2 concentration, as well as to dissolved oxygen. The latter may turn out to be an important screening criterion, as high DO is endemic to large systems. The response to pH and CO_2 determines the range of pH (given water alkalinity) in which a system can be operated. The pH should be as low as possible to obtain the highest dissolved CO_2 required for maximizing average production. But this must be balanced against outgassing loss of CO_2 which increases with concentration. Mixing speed affects the surface mass transfer coefficient.

The data obtained from one month of operation of a Scenedesmus culture with effluent recycle was used to evaluate the cost of the inputs to the system. Cell harvesting was inexpensively achieved due to the very good sedimentation properties of this strain which grew in large clumps. The nutrient, CO_2, and power inputs were monitored. The harvested biomass output averaged 15 $gm/m^2/day$. Initial input costs were \$1.09/kg AFDW and were dominated by CO_2 costs (75% of the total). Realistic assumptions concerning prices of CO_2 and nutrients, bought in bulk, led to a 75% reduction in projected cost. Another 50% reduction would be necessary and would have to come from the assumption of a source of CO_2 at below present market price. Increased productivity, although important in terms of distributing annualized capital costs and labor costs, has little impact on the inputs considered here since most are nutrients that are proportional to biomass output.

CONCLUSIONS

The major conclusions of this project can be summarized as follows:

1) Using two wildtype species in northern California a yearly average productivity of 15 $gm/m^2/day$, or 24 tons/acre/yr can be obtained in water with TDS = 4-8 ppt.

2) This can probably be increased to 20-25 $gm/m^2/day$ or 32-40 tons/acre/yr in southern California.

3) Productivity can probably be further increased by using competitive strains screened for

   a) low respiration rates
   b) tolerances to high levels of dissolved oxygen
   c) broad temperature optima
   d) resistance to photoinhibition.

4) In systems with randomized, turbulent mixing, productivity is independent of channel velocity at least for productivities up to 25-30 $gm/m^2/day$ and velocities from 1-30 cm/sec.

5) Storage product induction requires one to three days of growth in batch mode under N-depleted conditions.

6) Critical cost centers include CO_2 input, harvesting and system capital cost. Increased mixing speed increases CO_2 losses, power input, and system costs and must be adequately offset by very large increases in productivity.

7) Media recycling, necessary for water conservation, has no adverse effects, at least in the short term for strains which do not excrete organics, and when the harvesting method is at least moderately effective for all algal forms which may be present.

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SECTION 1.0

INTRODUCTION

1.1 BACKGROUND

This report is a continuation of a project performed by EnBio, Inc. under subcontract XK-3-03000-1. It was performed parallel to ASP species screening efforts and an outdoor project performed in Hawaii. The goal of the combined efforts is to advance the technology for producing lipid containing microalgae with the highest yields at the lowest cost. This specific effort was geared to identifying critical cost centers and elucidating methods for lowering their costs.

The project utilized both naturally invading algae and organisms provided from the species screening efforts of other ASP subcontracators. The media used in this effort spanned the range from fresh to brackish waters - 0.4 to 8.5 ppt total dissolved solids. The pond system used, built under the previous subcontract, consisted of plastic-lined 100 m^2 algal growth ponds, operated at 20 cm depth, and mixed by paddlewheel to provide slow to moderate channel velocities. Continuous and semi-continuous operational modes were tested, and batch nitrogen deprivation studied.

1.2 PROJECT GOALS AND TASKS

1.2.1 Longterm Productivity Data

One of the major tasks set forth for the project was the determination of yields ($gm/m^2/day$) averaged over an extended period of time. It was hoped that specifically screened strains, provided by other ASP subcontractors, would be used in generating the data. As it turned out, these strains did not compete well with indigenous algae, and only one, *Ankistrodesmus falcatus*, was successfully cultivated at the large scale for more than one month. It was grown for two months on two different occasions, each time it was eventually outcompeted by indigenous algae. Two species of the latter were cultivated for two months or more. A *Scenedesmus quadricauda sp* was grown for thirteen months and a *Chlorella sp* for two months. Most of the outdoor productivity data, and directed experimental results were obtained with these organisms. Much indoor data however, was obtained using the screened species.

1.2.2 Factors Affecting Yield and Species Dominance

Another major goal of the project was to try to learn what factors limited the productivities as measured and in addition, what factors were decisive in determining species dominance. Factors investigated in relation to

yield limitation included pH, CO_2 levels, dissolved oxygen levels, mixing intensity, temperature and light input. Species competition was studied in relation to tolerance to high and low temperature, high light, high DO, and mineral composition of the various growth media.

1.2.3 Critical Cost Centers and Potential Cost Reductions

Besides the obvious impact of increased productivity on cost, the operational variables were examined for their impact on the cost of biomass production. Thus mixing intensity was studied in terms of yield enhancement, power utilization, and CO_2 outgassing. Media recycling was examined since it is essential for water conservation. The physiology of CO_2 utilization, and its relation to pH and alkalinity, were studied. Sedimentation, unaided by chemical flocculant addition was investigated as a means of harvesting biomass. Although no concrete rules can be formulated, as strains vary in their requirements and characteristics (e.g. sedimentation rates, pH optima) specific costs can be attached to any procedure or operation aimed at increasing yield and hence its cost-effectiveness can be evaluated. The results of the analyses in this report were formulated to serve this kind of function.

1.3 ORGANIZATION OF THE REPORT

The following report is broken down into eleven sections including this introduction. In Section 2.0, materials and methods general to the report as a whole are outlined. However, some sections contain descriptions of those procedures which were specific to data collection reported therein. Section 3.0 presents the observational data obtained from extended cultivation of several strains. Thus, in addition to productivity data and correlations with climatic inputs, the characteristics of the organisms and the problems involved in maintaining prolonged cultivation are described. In Section 4.0 results are discussed from experiments that were aimed at taking a closer look at the physiology of yield dependence on factors like temperature, oxygen, light input and respiration. In Section 5.0 this discussion is extended to include mineral composition and salinity. Recycling of clarified culture effluents (water recycling) was also studied. Results from two six-week experiments are presented. In Sections 6.0 and 7.0 two of the most critical cost centers, mixing and carbonation are discussed in terms of experimental results. Two important operations, storage product induction and harvesting are treated respectively in Sections 8.0 and 9.0. Finally, all of the results are brought together in Selction 10.0 in an analysis of the operational costs of algal biomass production. Here the actual costs incurred during this project are summarized and from these, costs of production at the present state-of-the-art are projected for a large scale system. The major conclusions from the project are summarized in Section 11.0.

SECTION 2.0

METHODS

2.1 LABORATORY METHODS

2.1.1 Chemical Analysis

Dry Weights. Volatile suspended solids were concentrated either by filtration (washed with 4 x H_2O) or by centrifuging 10-35 ml at 17,000 rpm on a SS34 rotor in a Sorvall RC5B centrifuge (app. 20,000 xg) and discarding the supernatant. The filter or pellet was transferred to a preweighed weighing dish, heated at 103°C overnight, weighed, ashed at 550°C for fifteen minutes, and reweighed to obtain the volatile solids by difference. The data was calculated as mg/L of solids. For ash content the sample was either filtered through a Whatman GF/A paper and rinsed with 3-4 volumes of distilled water or the centrifuged pellet was washed with 10 ml distilled water.

Total Dissolved Solids (TDS). Total dissolved solids were determined by placing 10-15 mls of clarified culture (centrifuge supernatent) in a pre-ashed, pre-weighed aluminum weighing dish, drying overnight at 103°C, weighing, ashing at 550°C for fifteen minutes, and reweighing. The inorganic total dissolved solids were calculated as the difference between the final and initial weights.

Hardness [1]. 50 ml of sample (clarified culture or water) was placed in a 100-150 ml beaker, 0.5 ml EDTA-NH_3 buffer was added, then one scoop of indicator powder (0.5 g Eriochrome Black T mixed with 100 g AR grade NaCl) was added and the solution stirred. The mixture was titrated with EDTA titrant (3.72 g Na_2EDTA in 1 L distilled water standardized against a standard $CaCO_3$ solution) until the solution turned from obvious pink to obvious blue, but not purple. Hardness was expressed as mg Ca^{2+}/L or mg $CaCO_3$/L.

Alkalinity. Alkalinity was determined by titrating samples with standardized HCl or H_2SO_4 down to pH 4.20. The alkalinity was expressed as mmoles OH^- consumed per liter sample.

Generally a 100 ml sample volume was titrated with 0-20 ml 0.1N HCl. The solution was gently stirred with a magnetic stir bar, and the addition of acid was moderately rapid below pH 7 to minimize the effect of CO_2 escape.

Lipid Content [2]. Algal suspensions were concentrated by centrifugation. The pellet was used for both ash-free dry weight determination and lipid determination by the modified Bligh and Dyer method.

Total Carbohydrate Content [3]. 1.0 ml of 5% phenol solution was added to 1.0 ml sample containing 0.015 to 0.130 mg carbohydrate in an 18 x 150 mm tube. After mixing, 5.0 ml of 0.5% hydrazine sulfate in concentrated H_2SO_4 was added, covered, and mixed well with a vortex mixer. After cooling to room temperature, absorbence at 490 nm was determined and total carbohydrate concentration was calculated from a glucose standard curve.

Urea [4]. Clarified culture liquid was diluted appropriately and then treated with a buffered urease reagent to liberate ammonia. The ammonia was detected as below.

Ammonia. Ammonia was determined from clarified culture liquid using the phenol-hypochlorite reaction to form blue indephenol at high pH, according to the method of Solarzano [5].

2.1.2 Indoor Cultivation

1 Liter Roux Flask Cultures. A low light, continuously illuminated experimental apparatus was used for initial studies and bioassays. One liter Roux flasks (5 cm thick) were filled with 750 ml culture, sparged at 20-100 V/V/hr with a 98:2 air/CO_2, incubated at 28$\pm$ 2^{o}C at pH = 7.5$\pm$ 0.5. The bottles were placed in a light bank of cool-white fluorescent lamps at light intensities between 25-40 Wm^{-2} per side (see data tables for specific conditions). Productivities from these experiments were determined from ash-free dry weights and expressed as gm/L/day or gm/m^2/day, or percent visible light utilization efficiency.

1.4 sq. m Indoor Tank Cultures. A high light, diurnally illuminated experimental apparatus was used to produce innoculum for outdoor work, for comparative productivity data, and for media optimization and recycling experiments. Generally 1.4 m^2 tanks were operated as sequential batches at 10 cm depth (140 L) and mixed by paddlewheels (~1.5 fps). Light intensity varied depending on the experiment from 50-275 Wm^{-2} (see data tables for specific data) and was provided by two MS1000 metal halide lamps per tank. Temperatures were generally 25-30^{o}C. pH was adjusted by intermittent addition of 100% CO_2 controlled by pH regulators. Grab samples were removed daily at 1000 hours.

Sequential batches were operated by removing a percentage of the pond volume on a given schedule and replacing with an equal amount of fresh medium plus additional amounts of consumed nutrients in proportion to the biomass removed. When media recycling was practiced, a given volume of the pond was centrifuged through a Sharples T1 continuous separator, the effluent returned to the pond and consumed nutrients replaced.

2.1.3 Media Used

The cultivation media used varied with the organism being grown. Table 2-1 lists the media compositions.

Table 2-1. Media For Indoor Cultivation

	Scenedesmus sps mM	Chlorella Ankistrodesmus Oocystis mM	Isochyses mM
$NaHCO_3$	5.0-10.0	20.0	5.0-10.0
NaCl	4.0	56.2	400
$CaCl_2$	0.5	0.25	10.0
KCl	-	3.3	1.0
K_2H-KH_2PO_4 Buffer	2.0	1.0	0.2
Mg $SO_4.7H_2O$	2.0	-	24.0
$MgCl_2.6H_2O$	-	2.5	20.0
Na_2SO_4	-	1.5	-
$FeSO_4.7H_2O$	0.07	0.035	0.09
Na_2EDTA	0.14	0.07	0.18
Trace Soln	0.5ml/L	0.5ml/L	0.5ml/L
Urea	5-12	6-12	-
$NaNO_3$	-	-	12.0
B-12	-	0 or 0.5 ug/L	0.5 ug/L
Biotin	-	0 or 0.5 ug/L	0.5 ug/L
Thiamine	-	0 or 0.1 mg/L	0.1 mg/L
Na_2SiO_3	-	-	0.1
NaF	-	0.26	-
$Na_2B_4O_7.10H_2O$	-	0.26	-

Trace Soln:

Element	ppm	Element	ppm
Mn	0.5	V	0.01
Mo	0.1	Co	0.01
Zn	0.05	Ni	0.01
Cu	0.02	Cr	0.01
B	0.5		

2.2 OUTDOOR POND OPERATIONS

Outdoor ponds were operated as sequential batches, continuous cultures, and batch induction cultures. A schematic of the system is shown in Figure 2-1, and some photographs in Figure 2-2.

Dilution and Media All batch ponds were operated from initial inoculation or by emptying 75-90% of an operating pond culture and refilling with fresh medium. Density increased four to twelve fold prior to restart of the batch cultures. The basic medium components used were the same for all cultures and are shown in Table 2-2. Trace metals were available in sufficient amounts in the irrigation water used. Addition of chelated trace had no effect.

Continuous ponds were diluted automatically over a 12-hour period from 7AM to 7PM. Inflow was metered through a calibrated water meter. Outflow was via an overflow pipe and flowed by gravity to the harvesting ponds which were emptied automatically on a daily basis. Nutrients were fed to the ponds automatically from a nutrient mix tank (Urea, $NaHCO_3$, $FeSO_4$, EDTA) over a ten hour period or added manually each morning (P and Mg). Urea was added to reach a final concentration of 20-30 ppm urea-nitrogen.

Addition of Carbon Dioxide. CO_2 was added from 65 lb. tanks and, after 5/84 from a 6 ton tank via diffusers. Generally pH was kept between 7.0 and 9.5, occasionally it went as high as 10.0. Sodium bicarbonate was added to increase the CO_2 storage capacity. Alkalinity was measured to be between 5.0 and 20 meq/L. At 10 meq/L, approximately 80 mg/L of algal biomass can be grown during a pH rise of 8.5 to 9.5, excluding CO_2 loss due to outgassing. CO_2 additions were made automatic, slowly diffusing in CO_2 over an eight-ten hour period.

Sampling and Measurements Batch ponds were sampled manually (grab samples) each morning from four prescribed sampling points and composited. Samples were taken to the laboratory for solids, pH, and lipid analysis (if required). Effluents from continuous ponds were sampled every forty minutes from 9AM to 7PM using an ISCO compositor. Each night the compositor samples were collected and stored in a refrigerator until the next day. The compositor was not refrigerated. Measurements of fresh samples *vs* unrefrigerated samples indicated that on the warmest days as much as 15% of the biomass may have been respired away during compositing. Productivity values were not corrected for this, but recirculation of cooling water through the ISCO sampler was implemented starting 6/14/83.

Temperatures were recorded daily from max/min thermometers placed in each pond. Throughout, max/min ambient temperatures were recorded. pH was recorded manually twice a day.

A LiCor Solar Monitor automatically measured, recorded and stored daily solar input totals as watt-hour/m^2/day which was converted to Langleys per day.

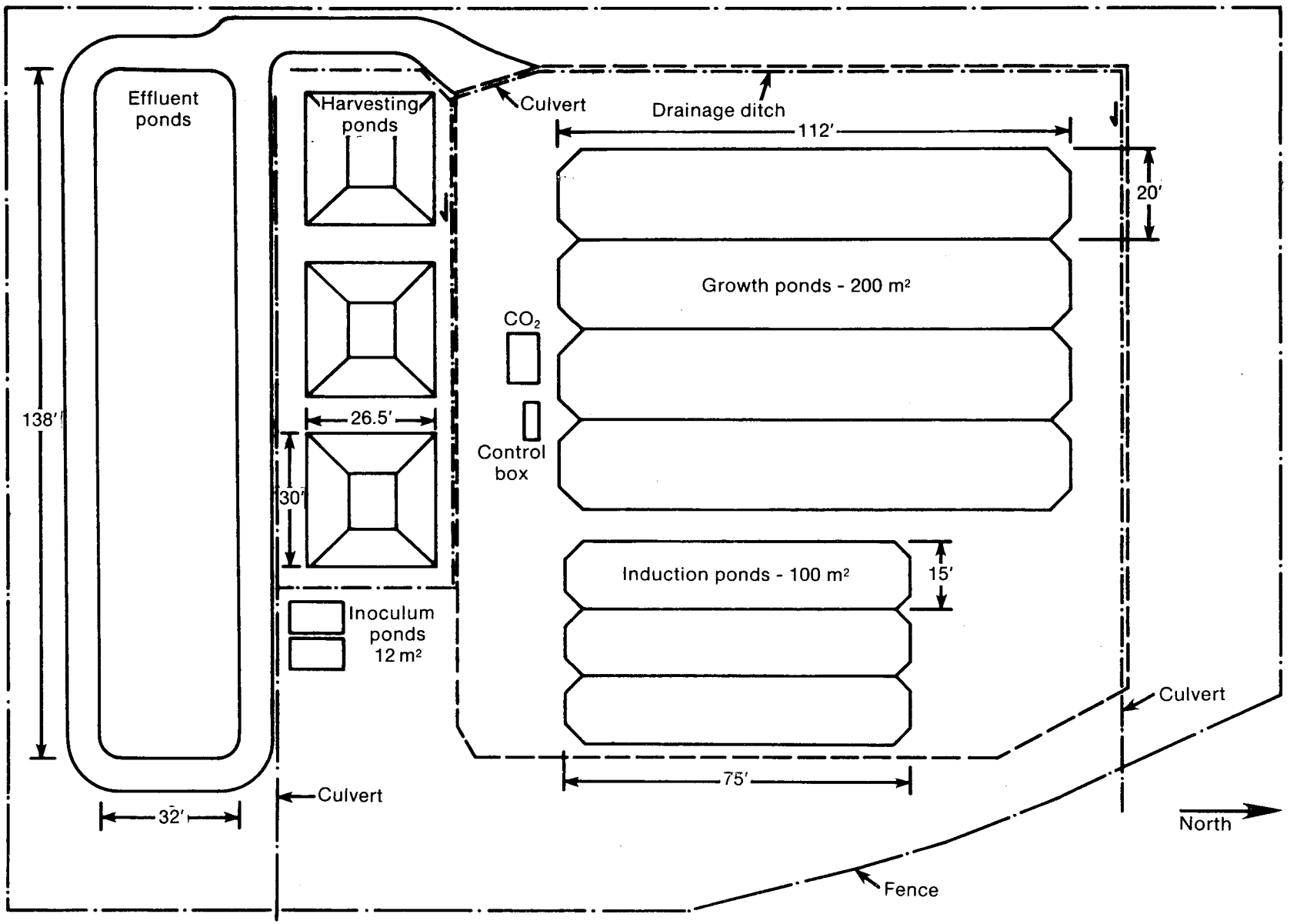


Figure 2-1. Pond System Layout

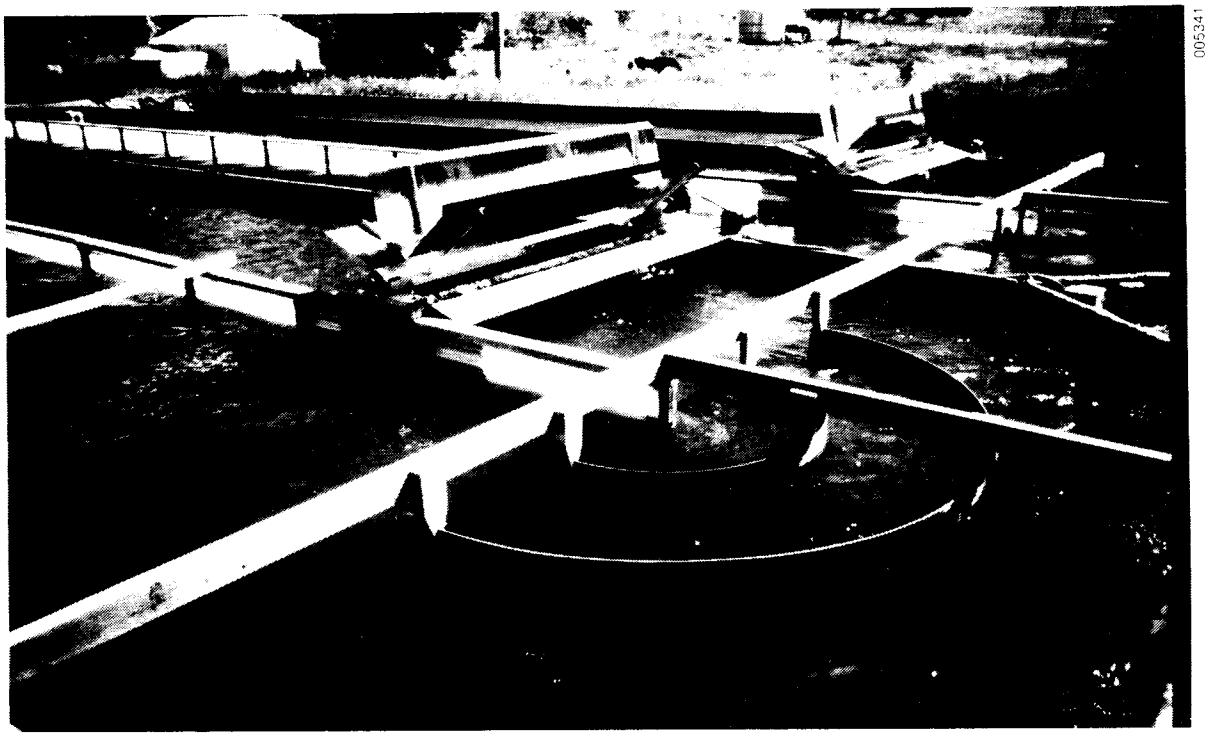


Figure 2-2. Views of the Pond System

Depths were measured daily and evaporative water loss recorded. Productivity for batch ponds was calculated using daily ash free weight differences (corrected for evaporative concentration) and expressed as gms ash free dry wt per m^2 per day. For continuously operated ponds, the weight of the composited sample (gm/L) was multiplied by the corrected outflow per day (L/day) and the product divided by the pond area (m^2).

Table 2-2. Media for Outdoor Cultivation

Nutrient Added	*Scenedesmus* spp mM	*Ankistrodesmus*, *Chlorella* mM
$NaHCO_3$	3.5	3.5-20
NaCl	0-112	56-112
Na_2HPO_4	0.2	0.2
K-muriate	-	3.3
$MgSO_4.7H_2O$	0.2	2.5
$FeSO_4.7H_2O$	0.1	0.1
Urea	0.35-0.70	0.35-0.70

Water Contains:
1.0-1.5 mM Alkalinity
0.1-0.2 mM K
0.5-1.0 mM Ca
1.0-1.5 mM Mg
0.2-0.5 ppm Fe
0.5-2.0 mM Na
0.5 mM Cl
0.2 mM SO_4

Trace:

B	0.2-0.3	mg/L
Zn	0.025	mg/L
Mn	0.03	mg/L
Cu	<.01	mg/L
Cr	<.01	mg/L
F	<.1	mg/L
Pb	0.012	mg/L
Hg	<.001	mg/L
Se	<.01	mg/L

2.3 SEDIMENTATION ANALYSIS

Sedimentation rates were measured using 500 ml graduated cylinders filled to 525 ml with well-mixed culture liquid. Samples were withdrawn from the 225 ml mark (15 cm below the 500 ml mark, 12.5 cm above the bottom) by siphoning through a thin line made of glass and plastic tubing. The line volume was about 6 ml. The density at the sampling point was measured in Klett units over a one hour period, starting at time zero, and then after 3, 6, 9, 15, 20, 30 and 60 minutes. In an idealized system the fraction of particles $(N_0-N_t)/N_0$ (N=Klett density), missing after a given time, say one hour, corresponds to the fraction of particles in the initial culture sample which have settling velocities greater than or equal to 15 cm/1 hr = 15 cm/hr. In order for this to be strictly true, several conditions must be met: (1) the initial mixture must be uniform, (2) the extent of the cylinder must be great enough so that accumulation of sedimenting (or floating) cells does not interfere with the further settling of cells, i.e., no bulking problems, (3) there are no other interactions which hinder removal, (4) no convection, (5) the particles must not flocculate prior to settling, and (6) the liquid level must not change significantly. None of these criterion can be rigorously met, except the first. The sampling point was taken near the midpoint of the cylinder to allow ample space for compaction (or bulking) of both floating and settling particles.

SECTION 3.0

EXTENDED CULTIVATION RESULTS

3.1 INTRODUCTION

Three species of algae were cultivated in 100 m^2 (and 200 m^2) ponds for at least one month. Two of the species were wild types that invaded the pond during cultivation of inoculated species. One, a Scenedesmus quadricauda invaded during freshwater cultivation of a laboratory grown strain, S. obliguus 1450. The S. quadricauda was cultivated for thirteen months in freshwater and for three months in brackish water (4 ppt TDS). In freshwater it was grown both continuously (continuous dilution over daylight hours) and semi-continuously (sequential batches diluted every three to seven days). During the winter of 1983-84 (Dec., Jan., Feb.) it was maintained in a 200 m^2 pond but no data were taken. Another wild strain, Chlorella sp. arose during attempts to grow Oocystis (Walker Lake isolate) in brackish water (4.5 ppt TDS), during July 1984. It was cultivated for two months, Aug.-Sept. 1984, semi-continuously. The S. quadricauda provided both long-term productivity data (this section) and, due to its pronounced tendency to form large settleable clumps, data on water recycling and biomass removal. The Chlorella, on the other hand, grew as non-settleable single cells which afforded the opportunity to measure productivity as a function of channel mixing velocity even at the very low end of the scale (Section 6.0).

Two strains, provided by species screening efforts of other subcontractors, were also cultivated. Ankistrodesmus falcatus was grown for two months at a time in the Fall of 1983 and Spring of 1984 in brackish water (4-8 ppt TDS). A freshwater Scenedesmus sp, So2a was grown during October 1983 both continuously and semi-continuously for a short period of time. However, neither of these strains could outcompete wildtype organisms in the long run. Two other pre-screened strains, an Oocystis and Chlorella So1a were introduced into the outdoor environment but could not be grown long enough to inoculate the large-scale ($\geq$100m^2) ponds.

Table 3-1 outlines the species grown at the mass culture level, the duration of cultivation, and the time of the year in which they were cultivated. In the data tables presented in this report, productivity is cited as both "sustained" and "maximum" productivities. Sustained productivities are averages over a specific period of time during which the cultivation was carried out, but it does not include data from very dilute suspensions (<50 ppm) or stationary cultures. Both of the situations arose often, as we tried to determine both the minimum and maximum densities which afforded good production. These changed with climatic conditions. Maximum productivity refers to an average of the maximum production value from each batch measured during the averaging period.

Table 3-1. Periods of Cultivation of Microalgae in $100m^2$, $200m^2$ Ponds

	1983						1984								
	J	A	S	O	N	D	J	F	M	A	M	J	J	A	S	O
S. quadricauda																
Freshwater semi-continuous	----------								----------							
Freshwater continuous	----------															
Brackish Water semi-cont.									---------							
Chlor. sp. Brackish Water Semi-cont.														------		
Ankistrodesmus Brackish Water semi-cont.	------			---					--------							
S. So2a Brackish Water semi-cont.				--												
S. So2a Brackish Water continuous				--												

3.2 SCENEDESMUS QUADRICAUDA WILDTYPE

3.2.1 Productivity Data and Culture Characteristics in Freshwater

Ten months of productivity data obtained from semi-continuous cultivation of S. quadricauda in freshwater are shown in Table 3-2 and Figure 3-1, along with climatic summaries. The sustained average productivity for nine months (excluding Nov. 1983 - February 1984 when productivity was about 3-5 $gm/m^2/day$) was 14 $gm/m^2/day$. Exclusion of one more month, July 1984, during which high temperatures eventually led to the death of the cultures, sustained productivity averaged almost 16 $gm/m^2/day$. Photosynthetic efficiencies, based on total insolation varied between 1.2 and 2.2%. The lowest efficiencies were recorded during the warmest months. Mid to late spring appeared to be optimal for cultivating this organism.

The cultivation media is listed in Table 2-2. The major chemical constituents from the irrigation water used are included; the minor constituents were also listed in Table 2-2.

Table 3-2. *Scenedesmus quadricauda* Productivity in Freshwater

Month/Yr	Average Productivity gm/m^2/d	Lgly/d	% Eff.	Maximum Productivity gm/m^2/d	Lgly/d	T, ^{0}C Min/Max	Typical Final Density ppm
Jul 83	15	658	1.2	18	664	20-32	275
Aug 83	14	568	1.4	17	584	18-32	300
Sep 83	14	413	1.8	18	504	16-29	300
Oct 83	13	327	2.2	16	343	12-22	300
Nov 83	5	158	1.6	6	248	5-13	250
Mar 84	13	419	1.6	15	411	9-22	400
Apr 84	15	456	1.8	17	549	11-26	500
May 84	20	656	1.7	26	671	17-31	800
Jun 84	17	673	1.4	25	688	20-32	500
Jul 84	10	600	0.8	15	673	25-37	400
Ave. 8 mo.	16						
Ave. 9 mo.	14						

The productivities from three months of continuous cultivation are depicted in Figure 3-2 and Table 3-3 and compared to concurrent semi-continuous values in Table 3-4. The difference in productivity was greater during the summer. Maximum pond temperatures were also lower during continuous cultivation as expected due to the significant dilution with water at 20-25^{0}C. Another important distinction is the maximal density achievable., i.e., the density typical at harvest time. Here semi-continuous (batch) cultivation is advantageous since densities were twice as great when productivity was reduced only 25%. As shown in Table 3-2, typical batch densities (and productivities) were even greater in 1984 than 1983 reaching 700-800 ppm at time of dilution.

A further distinction between continuous and semi-continuous operation is notable. The organism exhibited an extremely pronounced tendency to form colonies and for colonies to agglomerate into clumps. This occurred shortly after the initial invasive bloom (the lack of clumping upon initial blooming was observed three times). The colonies were predominantly four-celled, although two and eight celled colonies were present in significant numbers.

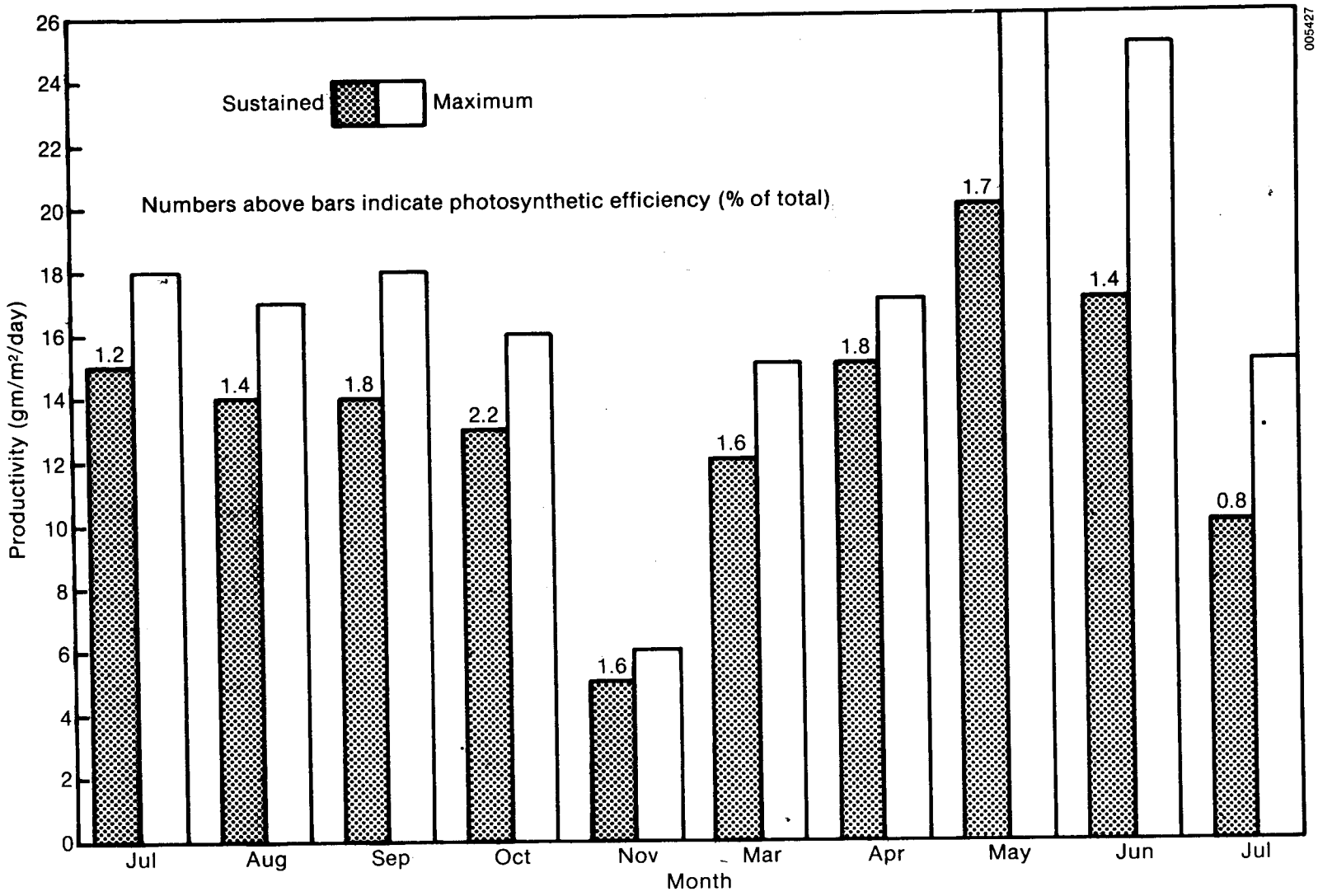


Figure 3-1. Longterm Productivity: S. quadricauda Freshwater

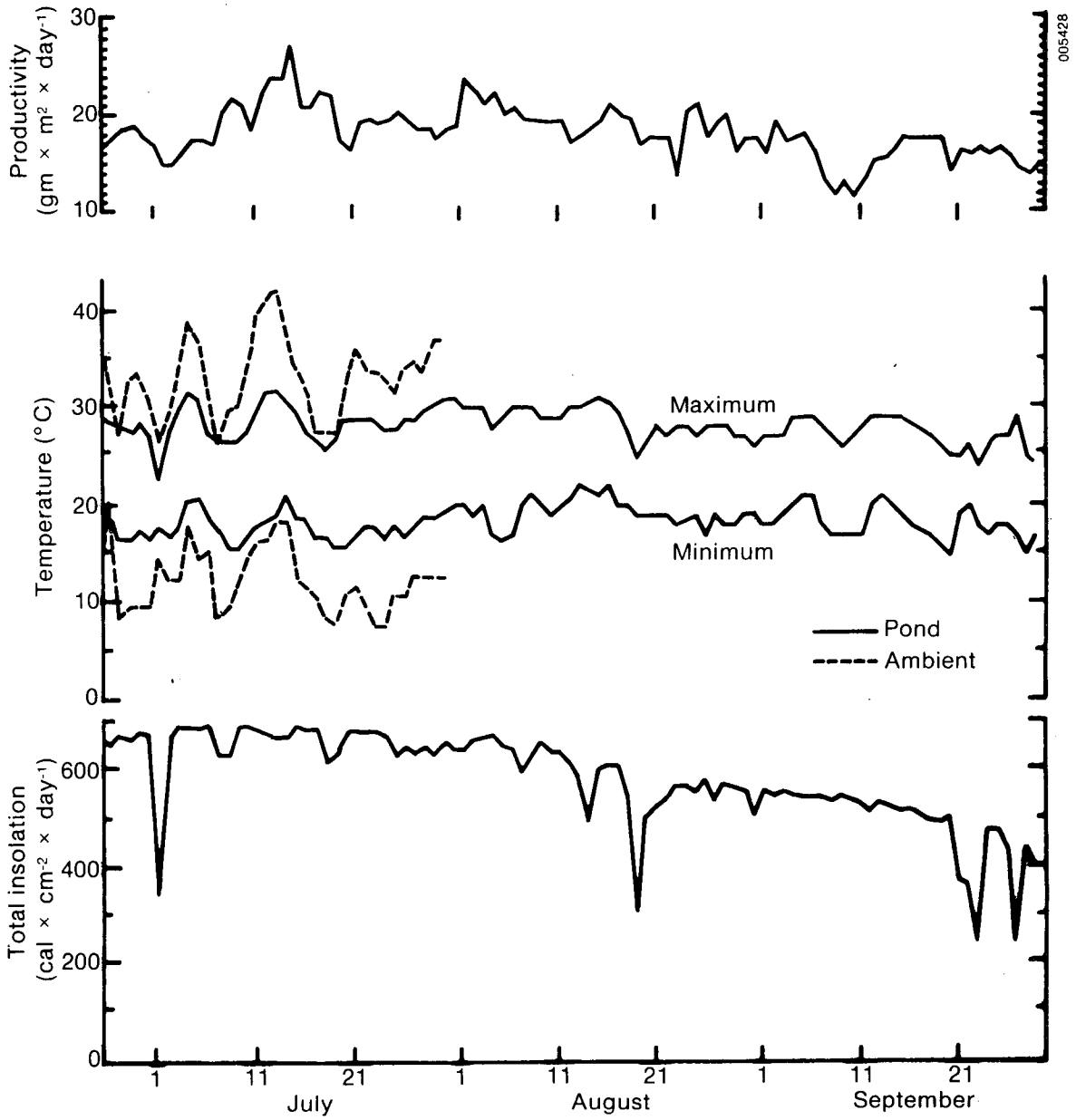


Figure 3-2. S. quadricauda Productivity - Continuous Dilution

Table 3-3. Scenedesmus quadricauda Continuous Culture Experiments

Dates	Det. Time days	Ave. Insol. Lgly/d	pH Range	Density ppm	Ave. Prod. $gm/m^2/d$	Ave. Eff. %	Max. Prod. $gm/m^2/d$	T°C Min-Max
6/26-7/15	1.70	645	7.5-8.5	115-200	17.2	1.2	19.1	18-29
7/16-7/29	1.86	665	8.0-9.0	135-155	20.4	1.4	27.2	18-28
8/1-18/31	1.7	580	7.5-9.5	115-170	18.8	1.8	23.2	19-28
9/1-9/6	1.9	550	8.0-9.5	113-160	16.7	1.7	18.7	19-28
9/7-9/11	2.6	538	7.5-9.3	134-154	12.0	1.2	12.6	17-27
9/12-9/29	2.7	483	7.2-9.3	160-180	15.4	1.9	16.9	18-26

Table 3-4. S. quadricauda Continuous vs. Sequential Cultivation

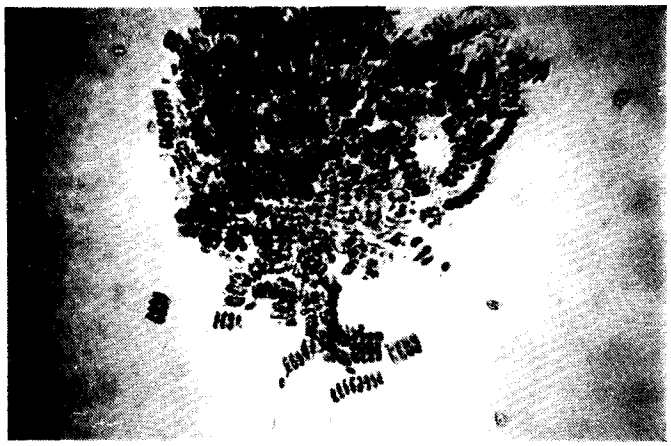
Month/yr	Cont. Prod. $gm/m^2/day$	Typical Density ppm	Seq. Batch Production $gm/m^2/day$	Typical Max. Batch Density ppm	Productivity % Increase
7/83	18.6	170	14.8	275	26
8/83	18.8	150	14	350	34
9/83	15	140	14	350	7

During continuous cultivation several colonies associated forming clumps of 20-100 um in typical dimension. However, during batch growth, hundreds of colonies clumped into flocs of one hundred to several thousand microns. Figure 3-3 shows a typical clump. Except during July 1984 when growth of the strain was tentative, these clumps were dense in cells and sparse in cell debris and detritus. It was typical that as temperature exceeded the optimum of 25-30^{o}C, more and more of the biomass of a clump was composed of cellular debris.

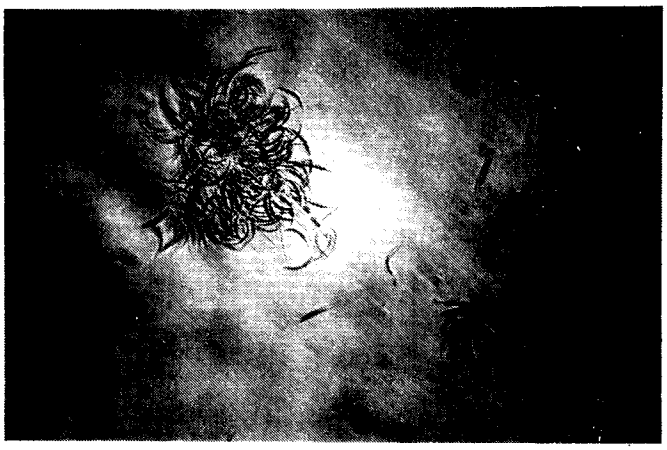
It was also observed that the S. quadricauda cultures were pleiomorphic and may not have been composed of one pure strain. Rather two morphologically distinct types of cells were always present, each in two size ranges. The dominant form resembled mature coenobia of S. quadricauda and comprised about 80% of the populations. It occurred in a large (5 x 13 um) and small (3 x 10 um) body size. The other form resembled reproducing S. quadricauda and occurred in 5 x 7 um and 4 x 6 um cell sizes.

3.2.2 Brackish Water

S. quadricauda was also cultivated in water containing 4.0 and 8.0 ppt TDS (March through May 1984), of composition shown in Table 2-2. This medium, although high in NaCl was low in both K^{+} and Mg^{2+} compared to the medium optimal for growth of other brackish water strains (Ankistrodesmus, Oocystis, Chlorella wildtype). Productivity summaries for 4.0 ppt growth are shown in Table 3-5, as weekly averages. In all cases productivities were similar. However, as will be discussed in Section 4.0, the temperature optimum was shifted downward as salinity increased.



A



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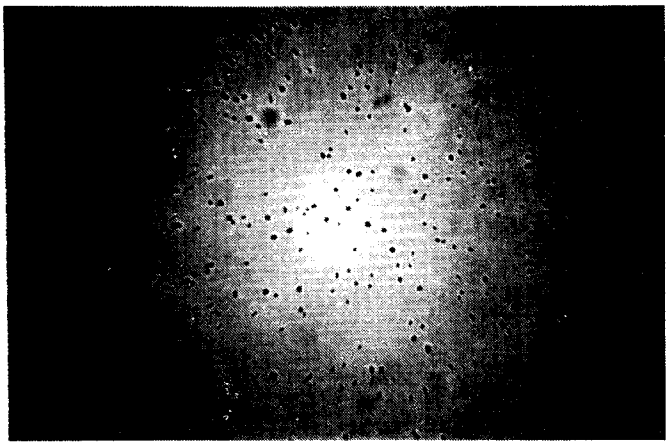

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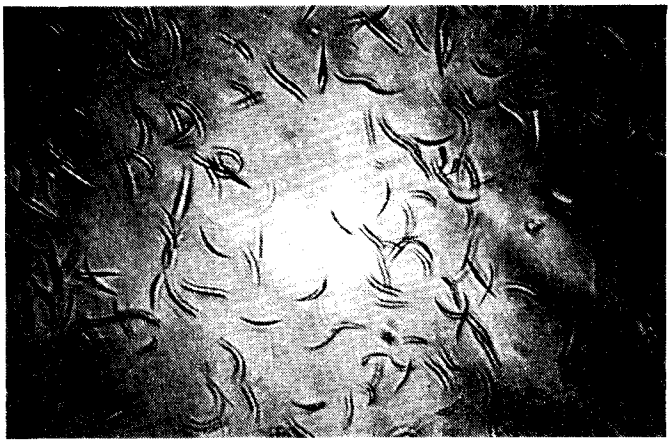

D

Figure 3-3. Photomicrographs of Algae Cultivated
A. S. quadricauda clump B. Ankistrodesmus clump
C. Chlorella sp. D. Dispersed Ankistrodesmus

Table 3-5. S. quadricauda Saline 4 ppt

Weekly Productivity Summaries

Mo./wk	Average Productivity gm/m^2/d	Lgly/d	% Eff.	Max Prod. gm/m^2/d	T,^{o}C Min/Max	pH	Density
4/1	13	456	1.5	16	11-22	8.6-9.5	370
2	16	532	1.5	19	8-25	7.6-8.6	680
3	10	476	1.2	15	13-29	8.5-9.0	960
4	17	601	1.6	20	13-24	8.2-8.5	740
	--						
	Ave=14						
5/1	18	561	1.8	23	13-23	8.0-8.5	940
2	19	655	1.7	26	14-27	7.5-8.5	750
3	15	590	1.4	18	12-29	7.8-8.4	550
4	14	669	1.2	20	15-30	8.0-8.3	550
	--						
	Ave=16						

3.2.3 Productivity Correlations

Table 3-6 presents another way of summarizing the freshwater sequential-batch growth of this organism. Here the values obtained for sustained productivity, maximum productivity and the environmental conditions of minimum pond temperature, maximum pond temperature, and total insolation are analyzed statistically. The data are from 39 sequential batches, not weighted by batch duration (as was done in previous productivity summary tables). It can be seen from Table 3-6 that the variance in the prediction of productivity is reduced about 50% by using a knowledge of any one of the three variables, in a linear regression. Adding more than one variable to the linear estimate adds little to the predictability of productivity. Better fits may have been obtained by, e.g., using two linear regions in a piecewise arrangement with a steeper slope at lower values of the temperatures and insolation. However, the sparseness of experimental points in this lower end renders such an analysis of questionable value. As will be discussed in Section 4.0, temperature optima seem to exhibit sharp cut-offs at the high and low end.

Table 3-6

A: Statistical Parameters of S. quadricauda Data: Sustained

Variable	Symbol	Mean	Variance	S.D.	n = 39
Productivity	P	14.7	15.8	4.0	
T_{min}	T_1	15.5	19.3	4.4	
T_{max}	T_2	27.8	28.6	5.3	
Total Insolation	I_T	526	26,600	163	

Multilinear Regression

$P_E = 6.71 + 0.42T_1 - 0.245T_2 + 0.0158\ I_T$

$R^2 = 0.555,\quad R = 0.745$

$S_E^2 = S^2(1-R^2) = 7.0$

Bilinear Regressions:

$P_E = 5.32 + 0.61T_1 \quad R^2 = 0.45,\ R = 0.67,\ S_E^2 = 8.7$

$P_E = 0.682 + 0.51T_2 \quad R^2 = 0.46,\ R = 0.68,\ S_E^2 = 8.5$

$P_E = 5.49 + 0.0176I_T \quad R^2 = 0.52,\ R = 0.72,\ S_E^2 = 7.6$

S^2 = Variance of productivity measurement

E = Estimate

S_E^2 = Variance of productivity about the regression line.

Table 3-6 (Cont.)

B: Statistical Parameters of S. quadricauda Data: Maximum

Variable	Mean	Variance	S.D.	n = 38
P	18.9	35.6	6.0	
T_1	15.9	17.1	4.1	
T_2	27.3	32.4	5.7	
I_T	535	26,658	147	

Multilinear Regression:

$$P_E = 5.7 + 1.26T_1 - 1.02T_2 + 0.0394\ I_T$$

$$R^2 = 0.615,\quad R = 0.784,\quad S_E^2 = 13.7$$

Bilinear Regressions:

$P_E = 3.6 + 0.964\ T_1$ $\quad R^2 = 0.44,\quad R = 0.67$

$P_E = -5.4 + 0.71\ T_2$ $\quad R^2 = 0.46,\quad R = 0.68$

$P_E = 3.3 + 0.029\ I_T$ $\quad R^2 = 0.51,\quad R = 0.72$

3.2.4 Summary

The wildtype S. quadricauda was the naturally competitive organism in freshwater and some brackish water formulations given the climatic conditions prevailing. Its natural tendency was to agglomerate into clumps. This tendency was most pronounced in freshwater semi-continuous operations, still pronounced in brackish water semi-continuous operations, and least evident in freshwater continuously diluted ponds. The clumping allowed easy harvesting. The longterm cultivation demonstrated that productivities of about 15 $gm/m^2/day$ longterm average should be easily obtained. However, the organism was not sustainable in the ponds at the warmest time of the year when maximum pond temperatures rose above 35^oC.

3.3 CHLORELLA WILDTYPE

3.3.1 Productivity and Culture Characteristics

The wildtype Chlorella was the naturally dominant organism during the summer in medium containing 4.5 ppt TDS, 2.5mM Mg^{2+} and 3mM K^{+}. Ankistrodesmus and Oocytis were the organisms originally intended to be grown in this medium. However, the Chlorella was only sustainable during the warmest time of the year. As temperatures dropped in October 1984 to below 15°C min. and 25°C max. the organism died.

Table 3-7 and Figure 3-4 show average weekly productivities measured with the Chlorella sp. Levels of dissolved oxygen were kept below those normally reached in the ponds. Section 6.0 has details. Sustained and maximum productivities were higher with Chlorella during the summer, than with S. quadricauda.

Table 3-7. Chlorella Productivity Summary

Mo/Wk	Average Productivity gm/m^2/day	Lgly/d	Eff. % Tot	Maximum Prod. gm/^{2}/day	Lgly/d	T,°C Min/Max	Typical Batch Ending Density ppm
8/1	23	641	2.0	30	641	19-32	560
2	22	610	2.0	31	552	21-32	390
3	18	601	1.6	23	615	21-32	325
4	22	525	2.3	26	536	19-30	625
	--			--			
Mo. ave.	21			27			
9/1	19	560	1.9	26	565	18-29	400
2	21	506	2.3	-	-	18-29	650
3	11	490	1.2	15	497	20-30	450
4	11	427	1.4	15	417	15-25	400
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Mo. ave.	16			20			

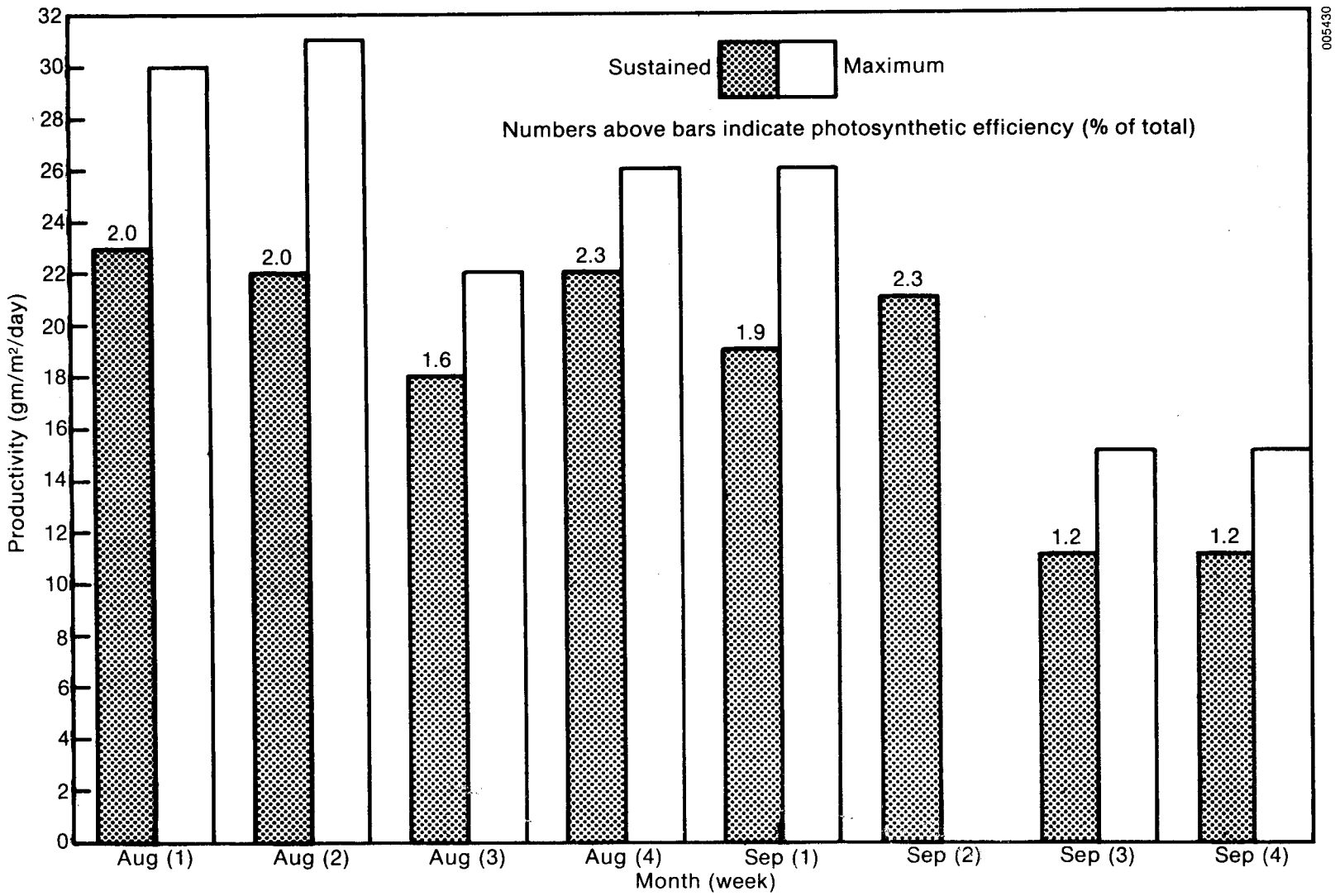

Figure 3-4. Longterm Productivity: Chlorella

3.3.2 Productivity Correlations

The data obtained from the Chlorella cultures were subjected to linear regression analyses as well. These data, however, were taken from a much more restricted period of time than the data from the Scenedesmus cultures. In addition, the Chlorella data were obtained during performance of a mixing speed experiment. Hence, operations and protocol were much more uniform in running the Chlorella cultures relative to the Scenedesmus cultures. As Tables 3-8A,B show, the correlation coefficients computed are much higher than those calculated for Scenedesmus. The average (sustained) productivity correlated only fairly with maximum temperature and poorly with total insolation. In fact, little decrease in the unaccountable variance of the predicted productivity is gained by adding knowledge of these variables to knowledge of minimum temperature. Of course, the variability of all parameters was fairly small (10-20% of the means) during these experiments.

The best correlations were found in the maximum production data set (Table 3-8B). Linear models explained most of the variability in productivity, mainly in terms of maximum temperature and insolation. About 80% of the variance could be accounted for by the changes in these variables. Partial correlations, however, did not indicate that the correlation between production and either maximum temperature or insolation was due to the linear correlation of either of these variables on the other.

The lack of correlation of some of the data may again be due to poor choice of model. A glance at the scatter diagrams (see Appendix) reveals that piecewise linear fits may have been a better choice of model for the sustained production data set and for the minimum temperature-maximum productivity data.

Better correlations were expected using maximum productivity data than average productivity. The latter includes points which were most likely limited by suboptimal cell density. Even the high correlation of maximum production with insolation and maximum temperasture is not evidence that no other variable limited productivity. Almost all productivity values were below 30 $gm/m^2/day$.

3.4 ANKISTRODESMUS FALCATUS

Ankistrodesmus falcatus was obtained from W.Thomas as a result of his species screening effort. It was cultivated during the late summer and fall of 1983 and again during the spring of 1984. Although the original medium recommended by Dr. Thomas called for vitamin supplementation, indoor experimental results indicated that this was not necessary. Consequently, after 9/28/83 vitamins were not included in the outdoor medium.

Table 3-8

A: Statistical Parameters of *Chlorella* Data: Sustained Production

Variable	Mean	Variance	S.D.	n = 18
P	18.7	21.6	4.6	
T_1	18.3	4.1	2.0	
T_2	29.0	7.8	2.8	
I_T	532	3,598	63	

Multilinear Regression:

$P_E = -15.2 + 2.38T_1 + 0.028T_2 - 0.02I_T$

$R^2 = 0.72$, $R = 0.85$

Bilinear Regressions:

$P_E = -16.3 + 1.91\ T_1$ $\quad R^2 = 0.695$, $R = 0.83$

$P_E = -17.0 + 1.2\ T_2$ $\quad R^2 = 0.547$, $R = 0.74$

$P_E = -5.06 + 0.045\ I_T$ $\quad R^2 = 0.366$, $R = 0.60$

B: Statistical Parameters of *Chlorella* Data: Maximum Production

Variable	Mean	Variance	S.D.	n = 14
P	23.5	40.7	6.4	
T_1	18.9	5.7	2.4	
T_2	29.3	10.2	3.2	
I_T	558	5,922	77	

Regressions:	Coeff. vector	R^2	R
P vs T_1	(-5.3, 1.53)	0.324	0.57
P vs I_T	(-15.4, 0.070)	0.704	0.84
P vs T_2	(-27.5, 1.74)	0.762	0.87
P vs T_2, I_T	(-26.1, 1.16, 0.028)	0.790	0.890
P vs T_1, T_2, I_T	(-25.4, -1.36, 2.07, 0.025)	0.867	0.93

Partial Correlations: Based on P vs T_{max}, I_T

$r_{PT.I} = 0.357$, I held constant

$r_{PI.T} = 0.375$, T held constant

Productivity data is summarized in Table 3-9. Productivity of this strain was lower than that of both wildtypes except in Nov. 1983 at low insolation and temperature. Until late Sept. 1983, the cultivation was characterized by a browning of the cultures after the first batch growth. It was determined (via bioassay) that insufficient potassium was the cause. Potassium deficient cultures greened quickly when brought indoors and supplemented with potassium. Outdoor brown cultures, after supplementation with potassium, greened very slowly and incompletely. The same problem with regreening occurred after nitrogen starvation.

The growth of this organism, when healthy, was characterized by a deep green color and a complete dispersing of cells. As any one of a number of nutrient deficiencies set it, the cells clumped (with scores of cells to a clump). The cells themselves were almost straight or sickle-shaped, with sizes varying between 12-40 u in length and 2-4 u in width (Figure 3-3). In addition to nutrient deficiency, clumping of cells was a consistent consequence of extended cultivation of this organism. The gentle mixing caused by paddlewheels appears to have enhanced clumping.

Another consistently observed characateristic of this organism was the tendency to bleach in high light or due to nutrient defiency. The bleaching, once advanced, was not reversible as was the case with the wildtype Scenedesmus strain. When dilute cultures were initiated under sunlight, this bleaching was typical, but chlorophyll content increased as density increased due to growth. Cultivation of this strain was limited both due to competition from wild strains (initially Selanastrum under low Mg^{2+} or K^{+} conditions, Scenedesmus or Chlorella otherwise) and to a temperature optimum lower than summer pond temperatures.

Average production from this strain was only 12 $gm/m^2/day$. It was difficult to prevent contamination by S. quadricauda during April 1984 and impossible during May 1984. Increasing the salinity from 4.5 to 8.5 ppt in April did not lead to faster growth, higher productivity or increased competitiveness. During the summer months of 1984, growth of Chlorella prevented the establishment of even a small-scale outdoor Ankistrodesmus culture.

3.5 SCENEDESMUS So2A CULTIVATION

A strain of Scenedesmus, designated So2a by its isolator (S. Lien) was grown for a short period of time in the 100 m^2 ponds. The duration of cultivation was limited by climate (cultures were inoculated in Sept. 1983) and contamination by the wildtype Scenedesmus. Table 3-10 summarizes the data. As the Fall progressed productivity declined. Since the organism was neither highly competitive, nor a lipid producer (Section 3.6) it was not studied further in 1984.

Table 3-9

Ankistrodesmus Productivity Summary: Weekly Averages

Mo/Yr,Wk	Average Productivity gm/m^2/day	Lgly/d	% Tot.	Max Prod. gm/m^2/d	T,°C Min/Max	pH	Density ppm
8/83,1	12	654	1.0	16	20-33	8.0-9.0	235-425
2	10.5	615	0.9	13.5	19-33	8.0-8.7	50-225
3,4	0	K^+ deficient					
9/83,1	13	545	1.3	13	20-30	7.2-8.5	65-330
2	14	526	1.5	16	20-31	7.9-8.5	167-306
3	10	456	1.2	14	16-29	7.8-8.5	178-420
4	15.5	402	2.4	18	17-27	7.6-8.8	75-300
Average	13			15			
11/83,1	6	120	2.8	8	7-16	8.5-9.2	100-290
2	7	210	1.8	9	6-14	8.5-8.9	150-300
3/84,1	11	430	1.4	11	8-19	8.8	260-460
2	8.5	375	1.2	12	10-21	9.0	170-250
3,4	12	485	1.4	16	12-22	8.3-8.8	80-380
Average	11			14			
4/84 1,2	12	460	1.4	18	10-24	7.9-9.2	140-600
3	12	544	1.2	18	10-29	8.2-8.4	100-800*
4	13	566	1.3	22	8-26	8.0-8.9	100-700*
Average	12			19			

* 2X salinity

Table 3-10. Scenedesmus So2a Cultivation: $100m^2$ 1983

Dates	Ave. Productivity $gm/m^2/day$	Lgly/d	Eff. % Tot.	Max.Prod. $gm/m^2/d$	T,°C min/max	pH	Density ppm	O days
				Batch Start-up				
9/27-29	10	355	1.5	16	15-25	8.0-8.5	35-195	
				Continuous Operation				
10/1-7	15	405	2.0	17	19-25	7.2-8-9	130-180	2.3
10/8-11	10	371	1.5	12	14-29	7.0-8.8	120-155	3.2

3.6 LIPID CONTENT

None of the strains tested in the outdoor system produced lipids in large amounts, either when N-sufficient or starved. Table 3-11 lists lipid content of the strains used outdoors.

Table 3-11. Lipid Content of Mass Cultures

Organism	N-Sufficient # tests	% lipid	S.D.	N-Starved # tests	% lipid	S.D.
S. quadricuada	8	20.2	2.4	2	13.8	0.1
Ankistrodesmus	9	26.3	3.2			
S.So2a	2	18.7	0.3			

3.7 CONCLUSIONS

- Productivities of 15-25 $gm/m^2/day$ were routinely obtained from wildtype strains throughout the 7-8 month "growing season." However, higher values were rarely seen. Thus a "barrier" of unknown origin appears.
- Continuous operation was about 20% more productive than semi-continuous operation, but "harvesting" densities were much lower.
- Pre-selected strains fared poorly in competition with wildtypes. If species screening is to be effective, more attention must be paid to the climatic conditions prevailing at the mass culture site.

SECTION 4.0

PHYSIOLOGICAL FACTORS AFFECTING NET PHOTOSYNTHETIC YIELD

4.1 INTRODUCTION

A noticeable aspect of the cultivation data is the consistency with which productivities were below 25 gm/m^2/day. In this section the following factors, which may have limited yield are discussed: grazing, temperature and levels of dissolved oxygen. It will be seen that each strain responds to a different extent to high levels of these parameters.

4.2 GRAZING, TEMPERATURE AND DISSOLVED OXYGEN IN PONDS

4.2.1 Overnight Losses and 1.4 sq.m Pond Results

Experiments were conducted to try to ascertain the reasons for low productivity in specific cases, and the appearance and disappearance of particular strains at certain times of the year. The S. quadricauda faltered during the hottest months, but dominated ponds at other times. The results below indicate that 1) this strain is respiratory at high temperatures, 2) that it is susceptible to grazing at high temperatures, i.e., when grazing activity increases, but 3) it is relatively tolerant to very high levels of dissolved oxygen.

Table 4-1 shows overnight respiratory and grazing losses measured in S. quadricauda cultures. Grazers were totally absent from ponds throughout 1983 and most of 1984. Only when pond temperatures reached 25°C overnight and 35°C during the day, for extended periods of time, did grazers bloom. This occurred in June 1984. The threat of grazing remained until Sept. 1984. During July-August 1984, even with heavy additions of aqua-ammonia, cultivation of S. quadricauda could not be maintained. The Chlorella cultures, on the other hand thrived, although each hatch needed to be operated on the final day, at high pH (>9.3) with 30 ppm free NH_3, for twenty-four hours, to eliminate grazing. As shown in Table 4-1 overnight losses of S. quadricauda biomass were absent in April when temperatures were low, and highest in June and July. The S. quadricauda clumps underwent a change from the end of May to mid-June from being densely packed with cells to being 50% composed of cell debris and detritus. Grazing increased during this period with typical rotifer levels of 0/ml in April, 0-1/ml in May and 10-100/ml in June. The overnight losses became nearly equal to daytime production when heavy grazing accompanied respiration. It should be noted that at low to moderate overnight loss (<10% of daytime gross) the precise value of the loss was difficult to resolve. The calculation involves subtraction of two large numbers (evening and morning dry wts.), each of which was subject to a 1-4% error. As overnight losses got large, these differences became more and more accurately resolvable.

Table 4-1. S. quadricauda Overnight Losses

Date	Gross Product. gm/m^2/day	Overnight Loss gm/m^2/day	Overnight Loss % of Gross	T^o,C min	T^o,C am	T^o,C max	Pond Size m^2	Rot. #/ml
4/25,26	15.1	+0.3	0±1	7	7	14	100	0
6/15,16	16.7	-0.3	0±1	15	16	25	1.4	0
6/24,25	21.8	-1.0	5±5	22	22	34	200	0
6/24,25	17.6	-4.0	23+6,-8	21	21	30	100	4
6/15,16	15.4	-4.0	26+13,-20	18	26	27	114	0
6/15,16	11.4	-1.4	12+30,-20	19	21	31	100	0
7/3,4	22.6	-21.2	95±10	24	29	37	200	100
7/3,4	20.0	-12.6	63±7	22	28	36	100	10
7/3,4	20.2	-15.2	75±8	22	28	36	100	10

Table 4-2 shows results fromm sequential batches in the 1.4m^2 ponds, one of which was heated. The collapse of the S. quadricauda occurred more quickly in the heated pond, which could not be grown to high density in late June. No grazers were present. By July when temperatures were above 35^{o}C, it could not be grown at all (no data shown). At this time temperatures in the unheated pond were also high. As can be gleaned from the table, higher temperatures first impacted production at high cell density.

Overnight losses for Chlorella are presented in Table 4-3. Here losses were low except in a heated 1.4m^2 pond, and in the 100m^2 pond (I-1) when overnight temperature was highest. At prevailing temperatures respiratory losses were 0-10%. Grazing increased losses and was most severe at higher temperatures. Productivity results from the 1.4m^2 ponds (Table 4-4) indicate that this organism tolerates temperatures from 12-15^{o}C at night and 25-38^{o}C during the day. Low respiratory losses were measured from Oocystis cultures as well (Table 4-5). The organism grew well in the 1.4m^2 ponds for short periods of time after inoculation (Table 4-6), but did not compete well enough to successfully inoculate the larger ponds.

Table 4-2. S. quadricauda 1.4m^2 Pond Results 1984

Dates	Days	T_{min} °C	T_{max} °C	pH	TDS ppt	Density ppm	Ave.Prod. $gm/m^2/day$	Max.Prod. $gm/m^2/day$	Heated
6/12-14	3	13	28	7.5	0.4	116-332	22	27	-
6/15-17	3	15	29	7.5	0.4	332-490	14	19	-
6/12-4	3	13	31	7.5	0.4	164-428	23	27	H
6/15-17	3	17	31	7.5	0.4	428-569	14	19	H
				BATCH #2					
6/19-21	3	13	28	7.75	0.4	133-298	15	18	-
6/22-23	2	13	28	7.90	0.4	298-400	14	16	-
6/1-21	3	18	31	7.75	0.4	110-308	18	20	-
6/22-23	2	18	31	7.80	0.4	308-250	-8*	-	H
				BATCH #3					
6/25-29	5	15	30	8.3	0.4	115-425	16	26	-
6/30-7/1	2	15	31	8.3	0.4	425-347	-8*	-	-

*Culture 50% dead

4.2.2 Productivity Profiles

In several instances productivity profiles were calculated in an attempt to determine how the biomass increase was distributed over the daytime. The first such experiment was carried out in April 1984 in a S. quadricauda culture (Table 4-7). Although production was not even over the day, afternoon cloud cover is easily seen to be the reason. The last row of the table gives an efficiency calculation, with errors. The errors are actual calculations based on the duplicate analysis of cell density. Thus although high efficiencies appear to have been obtained in the afternoon (and even overnight), the errors are so large that the values calculated are meaningless. Half-day averages shown in a later table indicate that in fact efficiency dropped in the afternoon. The data indicate that irradiance must be greater than 200 Wm^{-2} to obtain much biomass increase in a dense culture (>700 ppm). Pond temperatures were below 20°C, but even at low temperature and insolation, pond DO was quite high.

Table 4-3. Chlorella Overnight Losses

Date	Gross Product. $gm/m^2/day$	Overnight Loss $gm/m^2/day$	% of Gross	T, °C min	T, °C max	T, °C 1900	T, °C 0700	Rot. #/ml
			$1.4m^2$ unheated					
8/3,4	25.5	-1.6	6 +6-3	15	27	24	15	0
4,5	15.2	-0.8	5 +5-3	14	29	26	14	0
5,6	19.7	-0.8	4 +2-2	14	27	23	14	0
6,7	18.6	-1.9	10 +4-2	15	29	26	16	0
9,10	21.5	-1.1	5 +5-3	16	31	26	16	0
10,11	26.9	-3.7	14 +8-6	16	30	26	16	0
			$1.4m^2$ heated					
8/4,5	17.6	2.1	-	15	30	24	15	0
5,6	13.8	0	0	14	34	28	20	0
6,7	14.4	-6.6	46 +23,-23	16	34	29	23	0
9,10	30.8	-8.5	28 +5,-6	16	38	32	23	15
10,11	34.0	-8.5	28 +5,-6	16	38	35	23	30
			$100\ m^2$					
8/4,5	19.6	-2.0	10 +10,-5	19	31	30	19	0
5,6	30.8	-2.8	9 +5,-5	19	31	30	19	0
6,7	22	-4.0	18 +6,-9	21	32	30	21	15

Table 4-4. Chlorella: 1.4m^2 Pond Results

Dates	Days	T_{min} °C	T_{max} °C	pH	Ave.Prod. gm/m^2/d	Max.Prod. gm/m^2/d	Heated	Density ppm
7/23-25	3	15	30	8.3	34	40	H	108-493
7/26-27	2	14	31	7.5	26	-	H	130-323
7/28-29	2	15	31	7.5	18	20	H	323-461
7/30-8/2	4	14	31	7.2	23	30	H	113-461
8/8-11	4	16	38	7.5	20	31	H	101-398
8/8-11	4	15	31	7.5	19	23	-	95-381

Table 4-5. Oocystis: Overnight Respiration - 1.4m^2 Pond

Date	Gross Product. gm/m^2/day	Respiratory Loss gm/m^2/day	% of Gross	T,°C min	T,°C max	T,°C 1900	T,°C 0700
6/30-7/1	13.3	0	0 ±5	16	30	30	21
7/4,5	22.9	+0.5	2 ±2	19	32	31	19
7/4,5	23.7	-4.2	18 +6,-8	19	34	33	24

Table 4-6. Oocystis: 1.4m^2 Pond Results

Dates	Days	T_{min}	T_{max}	pH	Aver.Prod. gm/m^2/day	Max.Prod. gm/^{2}/day	Heated
6/27-30	4	16	31	8.5	22	30	H
7/1	1	16	33	8.6	0.6	-	
7/3-7	5	17	33	8.3	20	23	H
7/3-7	5	17	30	8.3	20	23	H
7/21-23	3	16	26	8.3	24	28	-
7/24-25	2	16	26	8.3	14	15	-

Table 4-7

Productivity Profile 4/25/84 *S. quadricauda* 100m^2 Pond
6 ppt

Time	0700	1100	1300	1500	1700	1900	4/26 0700
Temp, ^oC	8	12	17	18	17	14.5	7
DO							
% sat	160	---	450	400	310	235	-
ppm	18	--	40	37	28	23	-
Density							
ppm	660	698	721	726	732	734	736
±ppm	2	2	3	3	3	3	3
I_o, Wm^{-2}	226	978	981	303	224	003	261
I_T							
Langleys	16	208	378	488	523	539	551
% Total	3	38	69	88	95	98	100
differential	-	192	170	110	35	16	12
Productivity							
cum.gm/m^2	-	7.8	12.4	13.4	14.7	15.1	15.4
% Total	-	50	81	87	95	98	100
differential	-	7.6	4.7	1.0	1.2	0.4	0.3
diff. Prod. x10^3 / diff. Langleys		40±4	28±5	9±9	34±34	25±25	25±25

An afternoon productivity depression was observed on 6/13 and 6/15, 1984 in the 1.4m^2 ponds (Tables 4-8AB, 9AB). In these ponds mixing intensity (Wm^{-3}) was much higher than in the large ponds. This resulted in much increased gas transfer through the surface and hence lower DO levels. Pond temperatures were higher than in April. A disproportionate amount of the total yield was obtained prior to 1300 hrs. The effect was greater in the heated pond, as best evidenced from Tables 4-9AB, in which overnight losses are accounted for. Respiration is a possible cause, increasing as pond temperature increased. The effects of DO are uncertain, but as will be shown below, prolonged exposure to high DO only moderately reduced productivity of this strain in the laboratory.

Table 4-8A

Productivity Profile 6/13/84 *S. quadricauda*

$1.4m^2$ Pond - Unheated
0.4 ppt

Time	0700	1200	1400	(6/14) 0700
Temp, ^oC	12	22.5	26	12
DO				
%sat	100	240	280	100
ppm	10.6	21	21	10.6
Density				
ppm	182	207	226	240
±ppm	1	3	5	4
I_o, Wm^{-2}	0	906	906	0
I_T				
Langleys	0	266	426	698
%Total		38	69	100
differential		266	160	267
Productivity				
Cum.gm/m^2	0	6.7	11.7	15.4
%Total		44	76	100
differential		6.7	5.0	3.7
diff. Prod. $\times 10^3$ / diff. Langleys		25±4	31±14	14±9

Table 4-8B

Productivity Profile 6/13/84 *S. quadricauda*

$1.4m^2$ Pond - Heated
0.4 ppt

Time	0700	1200	1400	(6/14) 0700
Temp, ^oC	17	31.5	31	18
DO				
%sat	100	280	280	100
ppm	9.6	21	21	9.4
Density				
ppm	264	300	318	333
±ppm	2	3	1	3
I_o, Wm^{-2}	0	906	906	0
I_T				
Langleys	0	266	426	693
%Total		38	69	100
differential		266	160	267
Productivity				
cum.gm/m^2	0	9.7	14.5	18.4
%Total	0	53	79	100
differential		9.7	4.8	3.9
$\frac{\text{diff. Prod.}}{\text{diff. Langleys}} \times 10^3$		36±5	30±6	15±5

Table 4-9A

Productivity Profile 6/15/84 *S. quadricauda*

$1.4m^2$ Pond - Unheated
0.4 ppt

Time	0730	1300	1700	1900	(6-16) 0700
Temp, ^oC	14	27	28	27	14
DO					
%sat	140	240	180	-	100
ppm	14	19	14	-	10.2
Density					
ppm	332	372	-	395	394
±ppm	2	2		4	0.5
I_o, Wm^{-2}	261	927	546	180	159
I_T					
Langleys	17	336	623	683	691
%Total	2.5	49	90	99	100
differential	-	319	287	60	8
Productivity					
cum.gm/m^2	-	10.6	-	16.7	16.5
%Total	-	63	-	100	
differential	-	10.6	-	6.1	
diff. Prod. $\times 10^3$ / diff. Langleys		33±4		18±4	

Table 4-9B

Productivity Profile 6/15/84 *S. quadricauda*

$1.4m^2$ Pond - Heated
0.4 ppt

Time	0730	1300	1700	1900	(6-16) 0700
Temp, ^oC	25	31	31	29	23
DO					
%sat	130	240	185	-	100
ppm	10.6	18	14	-	8.5
Density					
ppm	428	469	-	486	471
±ppm	6	2		6	1
I_o, Wm^{-2}	261	927	546	180	169
I_T					
Langleys	17	336	623	683	691
%Total	2.5	49	90	99	100
differential	-	319	287	60	8
Productivity					
Cum.gm/m^2	-	10.9	-	15.4	11.4
%Total		71		100	
differential		10.9		4.5	-4.0
diff. Prod. x10^3 / diff. Langleys		34±7		13±8	

In Tables 4-8AB, the last row indicates that photosynthetic efficiency did not decrease at peak irradiance (1200-1400 hrs) but did decrease 50% thereafter. A halving of efficiency in the afternoon relative to the morning is again evident on 6/15 (Tables 9AB). Table 4-10 summarizes the split day profiles from S. quadricauda cultures (and one Ankistrodesmus culture). PM depression appears to correlate with pond temperature, with a greater decrease in efficiency occurring during warmest afternoons.

Table 4-10. Split-Day Productivities

				1st Half Photosynthesis			2nd Half Photosynthesis		
Date	T,°C			I_T	P	$(P/I_T)x10^3$	I_T	P	$(P/I_T)x10^3$
	0700	1300	1900	lang.	gm/m^2	gm/m^2 lang.	lang.	gm/m^2	gm/m^2 lang.
4/25	~8	17	14.5	362	12.5	34±2	161	2.6	16±6
5/7	14	23	29	332	10.2	31	332	9.4	28
	*14	24	29	332	8.2	25	332	8.0	24
	14	24	29	330	10.0	30	332	6.4	19
6/13	12	27	26	426	11.7	27±4	267	3.7	14±9
	x17	31	29	426	14.5	34±4	267	3.9	15±5
6/15	14	27	27	31.9	10.6	33±4	347	6.1	18±4
	x25	31	29	31.9	10.9	34±7	347	4.5	13±8

~ cloudy afternoon
* Ankistrodesmus culture. All others S. quadricauda
x Heated pond

PE = $(P/L)x10^3$ x (.055)

4-3. LABORATORY TEMPERATURE AND DISSOLVED OXYGEN EXPERIMENTS

Experiments were conducted in the laboratory in 1L Roux flasks both with strains cultivated outdoors and with strains of importance to the ASP. In this section, the effects of temperature and DO on net productivity are discussed.

Table 4-11 shows culture conditions and results of "control" experiments. Reproducibility of these experiments was good as evidenced by the small standard deviations relative to the means. The relative yields from cultures indoors was similar to outdoor results.

Table 4-11. Roux Bottle Cultures - Controls

Organism	pCO_2	Alk,mM	pH	T,°C	Wm^{-2}	$gm/m^2/day$	Eff,%	TDS,ppt	n
S.quad.	1	5	8.0±.3	30	20	13.5±0.5	12.0	0.4	2
Ankis.	1	20	8.2±.3	25	25	10.0±1.4	11.3	4.5	6
Isochysis	1	5	8.0±.3	25	25	8.0±0.5	9.1	28	5
Oocystis	1	20	8.3±.3	30	25	10.0±0.5	11.3	4.5	2
Chlorella	3	2-30	7.5-8.2	30	35	16.4±0.3	10.3	4.5	3

Figure 4-1 depicts laboratory results using S. quadricauda. The results are presented in order of descending productivity relative to the control. At 0.4 ppt TDS (freshwater), productivity was highest at 30°, reduced 20% at 25°C, and practically nil at 35° (data not shown: cultures were not sustainable at 35°C, constant temperature). DO in these air/CO_2 grown cultures was 100-125% of saturating. Increasing salinity to 4 or 9 ppt, reduced productivity 15% at 25°C, and also lowered the temperature optimum. Productivity was reduced another 15% at 30°C, 4 ppt relative to the control. Again, at elevated salinity and 35°C, cultivation was not sustainable. Sparging with O_2/CO_2 brought DO to a little above 500% of saturation. It reduced productivity over 20% at 30°C, 0.4 ppt but had little further affect on yield at elevated salinity and 30°C. Thus we can summarize by saying that only small effects were measured when salinity was increased and/or DO was high, and/or temperatures maintained between 25-30°C. The most relevant result is that the strain would not grow at 35°C.

Figure 4-2 shows results from Chlorella cultures. The pH effects will be discussed in the next section. With this organism, DO had a much greater effect, reducing productivity 50% during the first batch, 85% during growth of the culture after dilution, and (data not shown), killing the culture thereafter. However, switching back from O_2 to air, led to swift and almost total recovery. Figure 4-3 shows the same results with Ankistrodesmus. However, one important difference was the failure of this strain to recover when switched back to air. With both organisms, partial bleaching at pigmentation accompanied growth under pure oxygen. The Ankistrodesmus failed to "regreen" when switched back to air, as the Chlorella did.

Figure 4-4 shows a similar experiment with Oocystis. O_2 inhibition of yield was faster, occurring to greater extent during one cycle. This organism grew at 35°C but not at reduced pCO_2.

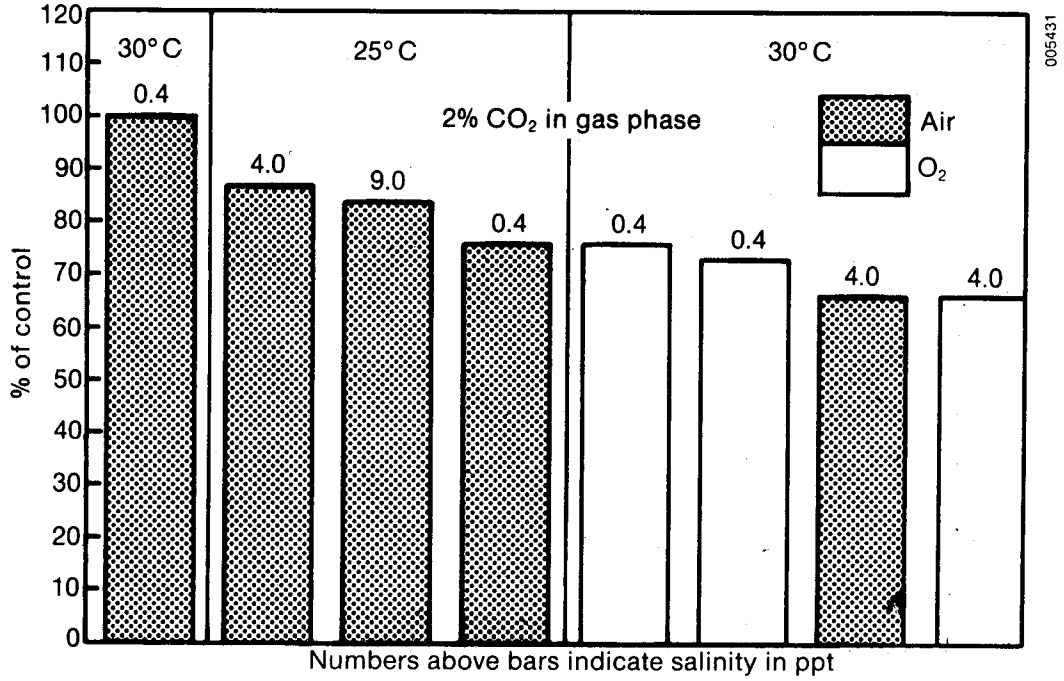

Figure 4-1. Scenedesmus: Productivity vs O_2, Temperature, TDS

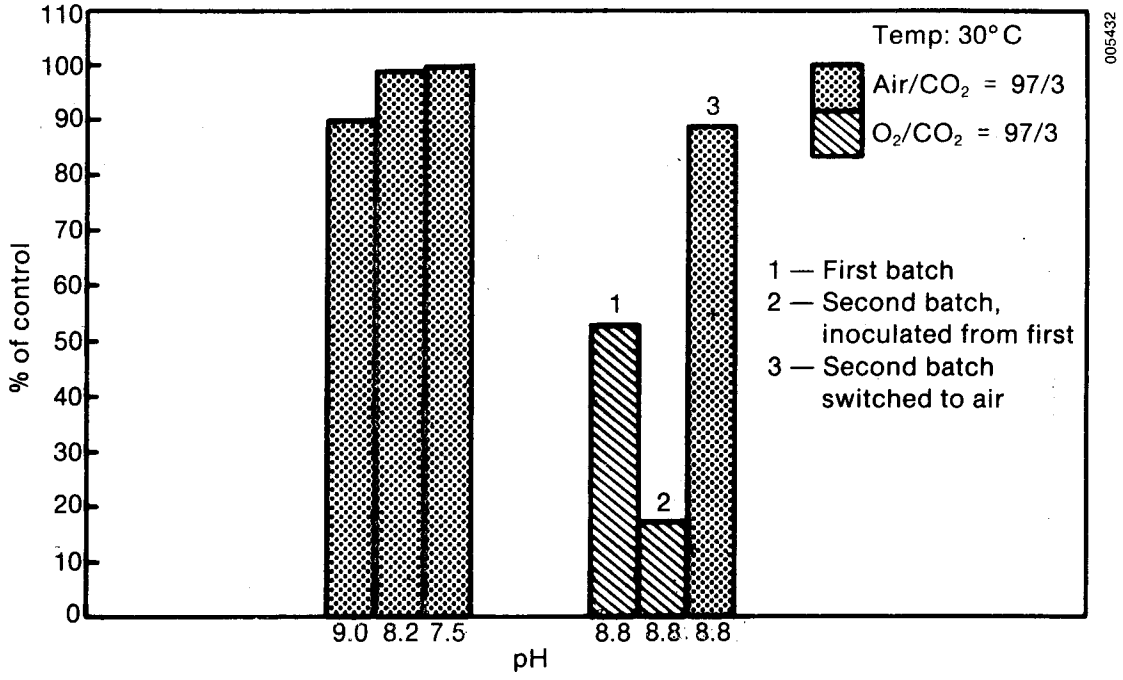

Figure 4-2. Chlorella: pO_2 - Productivity

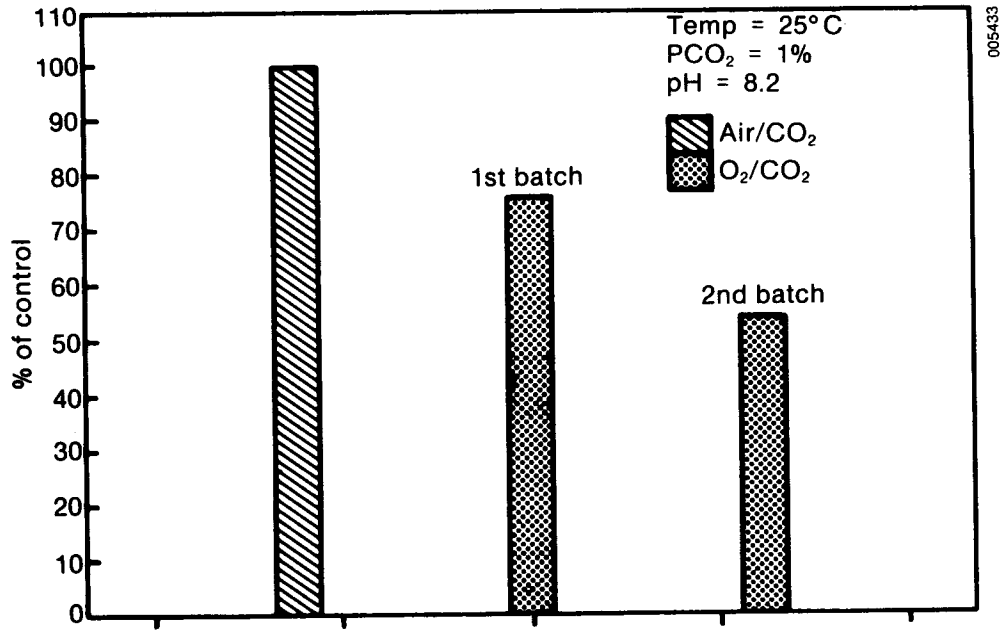

Figure 4-3. Ankistrodesmus: pO_2 - Productivity

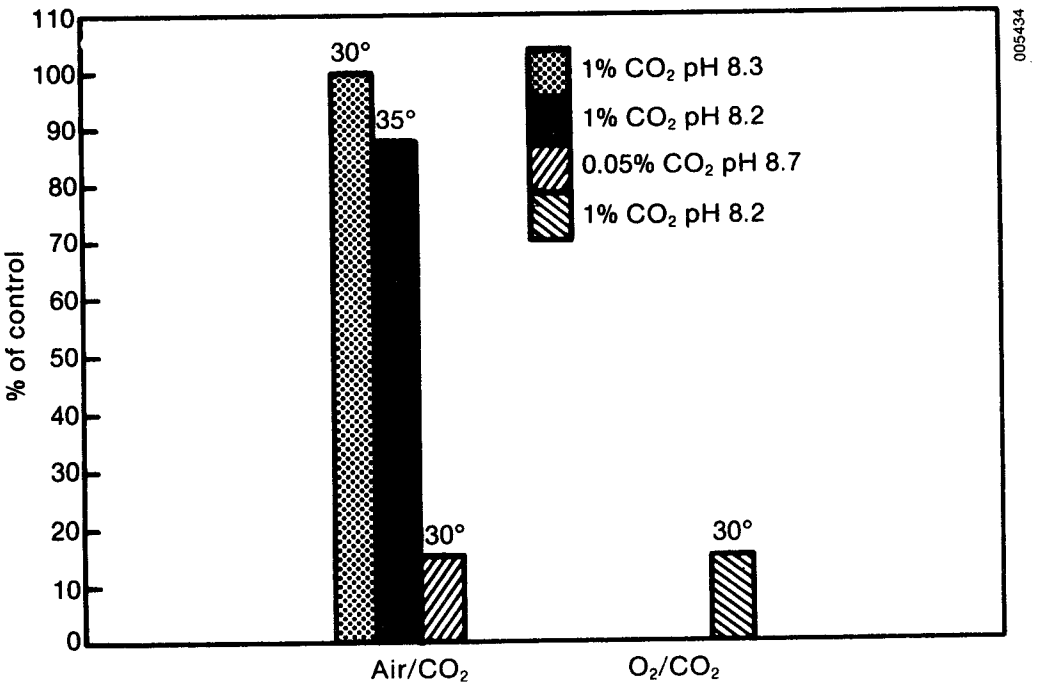

Figure 4-4. Oocystis: pO_2 - Productivity

4.4 CONCLUSIONS

The strains responded differently to various temperature and oxygen stresses. S. quadricauda tolerated high DO, but exhibited a temperature limit of around 30°C. In the outdoor ponds it grew well all year except when pond temperatures were highest in July and August 1984. The Chlorella tolerated high temperature (up to 40°C in outdoor 1.4m^2 ponds), was inhibited by constantly high DO, but recovered in fluctuating DO regimes. The Oocystis was greatly inhibited by DO. It was cultivatable in 1.4m^2 ponds (DO = 200% sat.) but not in 100m^2 ponds (DO = 500% sat.). The Ankistrodesmus was also sensitive to DO and exhibited poor ability to recover (resynthesize pigment) when switched from stressed to non-stressed conditions. Its performance in outdoor growth units was variable and subject to upset. It competed poorly.

Cultivating S. quadricauda at temperatures greater than 30°C led to an accumulation of dead cell debris in the clumps. This accumulation of detritus, which is known to be substrate for rotifer growth, along with higher rotifer activity at increased temperatures may explain the lack of resistance of the S. quadricauda to rotifer attack. The Chlorella was much less easily grazed upon.

Respiration and grazing appear to significantly limit net yield from some algae. High DO and photooxidation may also limit yields in mass culture. Screening under fluctuating conditions in which stressful conditions are realistically tested should eliminate strains that respire too much at high temperatures, exhibit low resistance to grazers (which may be related to high respiration rates), or are inhibited by high DO and light levels. Many of the pre-selected strains tested during this project did not tolerate high temperature, high DO, and/or high light intensity.

SECTION 5.0

SALINITY, MINERAL COMPOSITION, AND RECYCLING

5.1 INTRODUCTION

The mineral composition of the medium is an important factor in terms of species competition and suitability of strains in systems in which the composition may vary in time and space (i.e., well by well). Several of the strains were investigated for their tolerance to changes in total salinity and in composition of minerals. In addition two experiments were performed with recycling of clarified effluents to detect any short-term effects. One of these experiments used Ankistrodesmus in a $1.4m^2$ pond indoors. The other used S. quadricauda in a $200m^2$ pond outdoors.

5.2 TOTAL DISSOLVED SOLIDS TOLERANCE

S. quadricauda, Ankistrodesmus, and Isochrysis galbana (UTEX 987) were tested for tolerance to different levels of overall salinity. Each displayed tolerance to the levels tested, however this did not guarantee a high level of competitiveness at each TDS level. For instance, it was difficult to culture Isochrysis in open, $1.4m^2$ tanks at 7 ppt TDS due to the rapid invasion by Ankistrodesmus. Of course the low productivity of Isochrysis even at 28 ppt would result in a saltwater strain taking over in intensive open culture.

Table 5-1 shows mass culture results from S. quadricauda and Ankistrodesmus cultures during the spring of 1984. The Scenedesmus grew about as well at TDS 4.0 as in freshwater, and somewhat less well at 8.5 ppt. As was shown in Section 4.0, however, its high temperature tolerance decreased as salinity increased. This was evidenced in the outdoor work by the order in which culture productivity decreased with time, leading to subsequent loss of the culture. As summer arrived, first the 8.5 ppt culture died, then the 4.0 ppt culture and then the freshwater culture. The Ankistrodesmus grew as well at 4.0 ppt as 8.5 ppt, but could not outcompete the Scenedesmus at either. It took over both Ankistrodesmus cultures within four weeks.

Salinity tolerance of Isochrysis is shown in Table 5-2AB. n represents the number of sequential batches. At all TDS levels, photosynthetic efficiency dropped as irradiance increased. The closed, axenic 1L cultures were relatively easy to keep going. However, lag times upon reinoculation of each successive batch were long unless initial densities were kept high ($\geq$250 ppm). The open, $1.4m^2$ ponds were very difficult to operate due to lags, erratic growth, and contamination.

Table 5-1. Productivity and TDS - 100-200m^2 Ponds, 1984

Month	Organism	0.4 ppt	4.0 ppt	8.5 ppt
March	Scenedesmus	13	11	-
April	Scenedesmus	15	14	-
	Ankististrodesmus	-	12	12.5
May	Scenedesmus	20	16	13

Table 5-2A. Isochrysis Productivity and TDS - 1.4m^2 Ponds

	TDS	Aver. Prod. $gm/m^2/day$	Eff. % Vis	Max. Prod. $gm/m^2/day$	Max. Density ppm
n = 2	28	6.3± .8	2.8	8.1±1.1	550
n = 2	7	6.9±1.7	3.0	9.8±0.4	700

I_o = 125 Wm^{-2} = 125 Langleys/day, L:D::12:12

Table 5-2B. Isochrysis Productivity and TDS - 1L Cultures

	TDS	Prod. ±SD $gm/m^2/day$	Eff. % vis	I_o Wm^{-2}
n = 9	28	8.6±1.4	9.1	25
n = 7	21	8.3±2.1	8.8	25
n = 9	7	5.8±0.9	6.1	25
n = 13	28	11.8±6.4	7.8	40
n = 12	21	12.0±6.5	7.9	40
n = 13	7	7.5±2.1	5.0	40

The maximum density that 1.4m^2 pond cultures of Isochrysis reached was less than that of other algae grown under similar conditions. Data were taken to measure loss of biomass during the twelve hour dark period, and excretion of products into the medium. Table 5-3 shows these data from an Isochrysis culture (28 ppt TDS, 125 Wm^{-2} illumination), and an Ankistrodesmus culture for comparison. Both nighttime respiration and excretion of metabolic products were much greater in the I. galbana culture. Both cultures were grown under N-sufficient conditions. When particulate biomass (ash-free) of I. galbana reached 400 ppm, the dissolved levels of protein, CHO and TOA (total organic acids) all together were more than 40% of that. During this time, along with production of 100 ppm of particulate biomass, almost 60 ppm of carbohydrate and organic acids were excreted into the medium. As shown in Table 5-3, no measurable protein was excreted by either organism. Although extracellular levels of carbohydrate, produced by I. galbana constantly increased, organic acid levels increased and decreased with time.

Table 5-3. Losses From 1.4 m^2 Pond Cultures

	Ankistrodesmus (125 Wm^{-2})	I. galbana (125 Wm^{-2})
Production during light period	16.2	4.7
Loss during dark period*	-3.6	-2.2
Net 24 hr production*	12.6	2.5
Biomass density, VSS ppm	600	400
Dissolved protein, ppm	0	0
Dissolved CHO, ppm	2	60
Dissolved organic acids, ppm	2-30	90-115
TOA/Biomass	0.01	0.40
CHO/Biomass	0.01	0.20
Protein/Biomass	0	0

*$gm/m^2/day$

5.3 MEDIUM COMPOSITION AND CULTURE REQUIREMENTS FOR ANKISTRODESMUS

The Ankistrodesmus strain was grown in 1.4 m^2 tanks to determine the requirements for adequate growth and thus the cost - productivity trade-offs for media constituents. The data obtained indicates that expensive media inclusions, such as trace metals, EDTA, and alkalinity need not be added in large amounts. Indeed trace requirements can be supplied by the water resource, EDTA is necessary in only small amounts (<10 uM) or not at all, and alkalinity tolerance is broad (at least 5-20 mM). Problems were encountered in attempting to study long-term growth of this organism: as semi-batch cultivation endured the culture degenerated, losing its productivity and competitiveness and as light intensity was raised to 40% solar, this deterioration accelerated.

The first series of experiments compared growth at several light intensities and pH regimes. Table 5-4 shows productivity data vs. light input and pH for healthy, dispersed cultures. From the table it is evident that cultivation at pH 7 resulted in the highest productivity. However cycling pH between 6.5-9.5 was nearly as satisfactory. Productivity was lower at pH 8.7. Figure 5-1 illustrates productivity vs. light input for healthy, dispersed cultures at optimal pH and at temperatures equal to 26±2°C. The average productivities (averaged over the entire batch cycle) show the saturation that would be expected in a turbulent, but not intensely mixed system (mixing speed approximately 45 cm/sec, 1.5 fps). Maximal productivities (averages of the maximal production of each batch) show this to a lesser extent with basically a steeper linear increase at low light vs. high light. This leveling of productivity vs. light input became very pronounced for both average and maximum productivity as the irradiance was increased above 200 Wm^{-2}. In addition, clumped Ankistrodesmus cells competed very poorly with contaminants at the higher light intensity. Table 5-5 shows productivity data as a function of media composition. Lowering EDTA to 13 uM (.2 mole EDTA : 1 mole Fe) from 130 uM (2 mole EDTA : 1 mole Fe) had little effect. It might not be expected that such a short-term lowering (1 sequential batch) would have much effect on availability of trace metals, but even over the long-term (5 sequential batches) lowering the EDTA did not lower productivity in comparison to a long-term control (Table 2-1). Since iron was near yield limiting levels in the medium (4 ppm as Fe for 1500 ppm biomass), even short-term reduction in EDTA, if important, should have had an effect.

Table 5-4. Light Intensity - pH: Ankistrodesmus

n	Langleys	Avg Wm^{-2}	Avg. Prod.	Avg. Eff.	Max. Prod.	pH	Media	Cond.
4	80	60	12(10)	8.2	14(12)	6.5-9.5	Basal	D
3	80	60	8(7)	5.5	14(12)	8.7	Basal	D
4	120	100	16(14)	7.3	23(20)	6.5-9.5	Basal	D
1	120	100	19(16)	8.7	23(20)	7.0	Basal	D
4	120	100	14(12)	6.4	19(16)	8.7	Basal	D
2	125	125	15	6.6	20	7.0	Basal+	80% D
2	175	175	20	5.5	30	7.0	Basal	D
1	225	225	20	4.9	25	7.0	Basal+	D

See Table 5-5 for media used. D = dispersed.
Values in ( ) were normalized from L:D cycle of 14:10 to 12:12

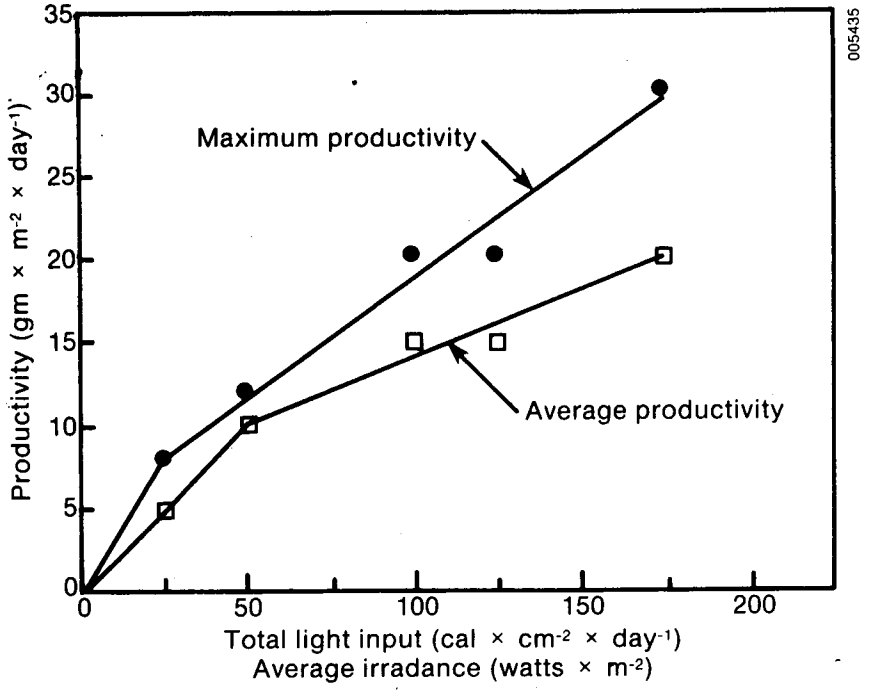

Figure 5-1. Ankistrodesmus: Productivity vs Light Input

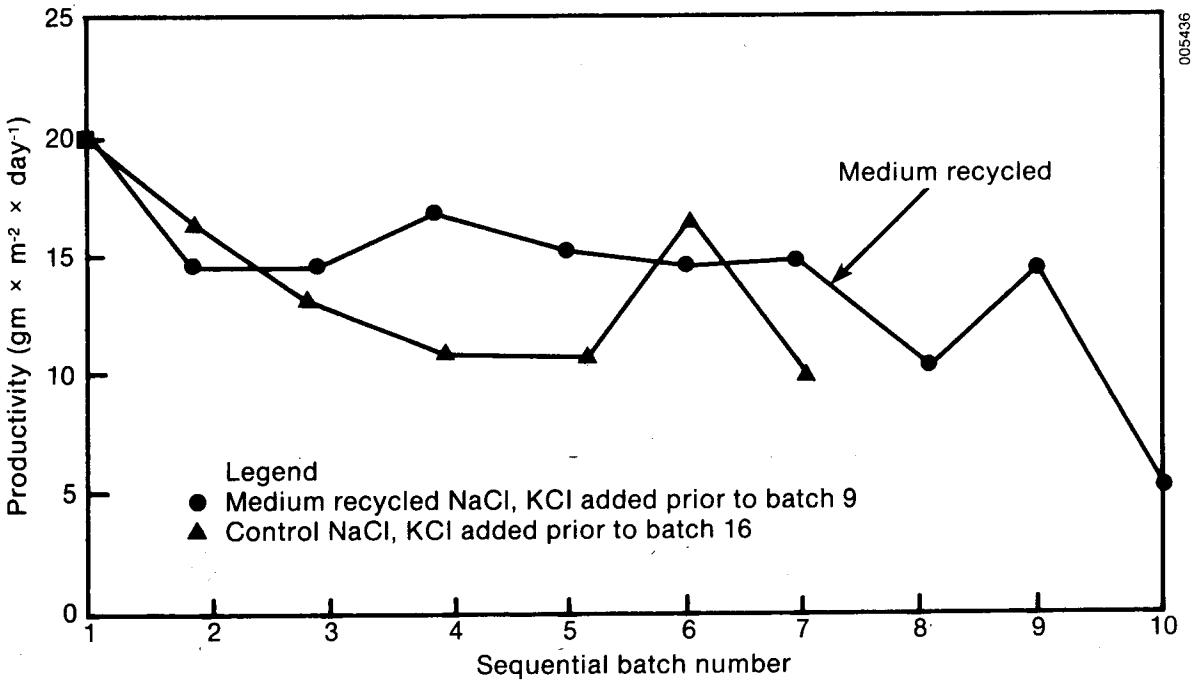

Figure 5-2. Sustained Sequential Batch Growth of Ankistrodesmus

Table 5-5. Ankistrodesmus: Medium Composition Experiments

Sequen. Batch	Lang./day (Wm^{-2})	Ave.Prod. $gm/m^2/day$	Ave.Eff. % vis	Max.Prod. $gm/m^2/day$	Medium	Culture Condition*
			EDTA Experiments			
Initial:					Control:	
1	175	20	6.2	30	125um Na_2EDTA	D
1	175	20	6.2	28	13uM Na_2EDTA	
Long-term:					Control:	
2-6	175	14.2	4.4	20.8	125uM	75%C
2-6	175	13.6	4.3	17.4	13uM	75%C
			N-Addition Experiments			
1	175	16	5.0	20	Control	25
1	175	15	4.7	21	Dosed Urea-daily	25
1	175	12	3.8	22	Dosed NO_3 daily	25
			TDS Experiments			
1,2	175	15	4.7	25	Control	90%C
2	175	15	4.7	21	2x salts, Urea	90%C
1	175	12	3.8	22	2x salts NO_3	90%C
			Trace Metals Experiment			
1,2	125	15	6.6	20	Control	D
1,2	125	14.3	6.3	19	10% trace	D
1	125	13.0	5.7	17	4% trace	D

*D = dispersed C = clumped

Elevating boron and floride levels (to levels in Pyramid Lake from which the Ankistrodesmus was isolated) also showed no difference from the control. Bioassays also indicated that low levels of these elements were not responsible for culture deterioration. Adding urea incrementally (1-2 mM per day) was no better or worse than adding it all at once (10 mM urea-N initially). Nitrate could be substituted for urea. Doubling the levels of NaCl, KCl, $CaCl_2$ and $MgCl_2$ had no short-term effect. Neither did lowering only Mg levels. Growth of cells which were obtained from the outdoor ponds and thus grown for a prolonged period of time on trace metals available in the water supply only (10-50% of lab levels) was as good as controls. Reduction of alkalinity levels from 20 mM to 5 mM did not change productivity. In the short term Ankistrodesmus grew equally well over a range of salinity, mineral composition, alkalinity, trace metal and chelator concentrations. In addition, growth in outdoor ponds and at low

light (30 Wm^{-2}, continuous) with and without vitamin supplementation indicated that vitamins are not required for productivity up to 20 $gm/m^2/day$.

As culture degeneration appeared to be a serious problem, data was obtained to document it and bioassays were performed (under low lights in 1 L bottles) to determine whether it was reversible.

Decreases in productivity were associated in every instance with cell aggregation into clumps. As indicated in Table 5-5, as clumping increased, productivities of control cultures decreased. Eventually the cultures failed, i.e., productivity declined precipitously and contaminants grew. These events became typical. No significant levels of extracellular products were measured.

Table 5-5 (Continued)

Sequen. Batch	Lang./day (Wm^{-2})	Ave.Prod. $gm/m^2/day$	Ave.Eff. % vis	Max.Prod. $gm/m^2/day$	Medium	Culture Condition*
			Alkalinity Experiments			
1-2	125	15	6.6	20	20mM Alk (Control)	D
1-2	125	15.6	6.8	21	5mM Alk	D
1	125	13.3	5.8	15	1.5mM Alk	
			High Light Intensity Experiments			
1	225	18.6	4.5	23.5	5mM Alk (Control)	Good
2	225	13	3.2	21	5mM Alk	Bleaching
3	225	Dead	---		5mM Alk	
1	225	14.4	3.5	23	10mM Alk (Control)	Good
2	225	16.3	4.0	22	10mM Alk	Bleaching Clumping, Debris
3	225	Dead			10mM Alk	
			Magnesium Experiments			
1	225	16	3.9	24.4	10mM Alk, 0.5mM Mg	Good
2	225	14.3	3.5	22.6	10mM Alk, 0.5mM Mg	Bleaching Clumping, Debris
3	225	Dying	---		10mM Alk, 0.5mM Mg	

A new culture of the Ankistrodesmus_ was obtained (from Dr. W. Thomas) and grown. It was, as before, initially mostly dispersed. When grown at 25 Wm^{-2} continuous illumination, the cultures became about 20-30% clumped. However, when grown at 150-250 Wm^{-2} with a 12 hr. light cycle, the cells became more clumped as the number of sequential batches increased. The same medium was used in both cases. Thus, it appears that high light intensity may be responsible for deterioration of the culture. Cultures grown at 225 Wm^{-2} deteriorated faster and further than those grown at lower light intensity (Table 5-5). By the second batch, bleaching was evident and by the third batch the cultures were dead. In order to determine if a nutrient deficiency was also involved in the culture deterioration, bioassays were performed. The bioassays consisted of (1) growing healthy (dispersed) cells in basal laboratory medium, outdoor pond water, and clarified liquid from an unhealthy pond; (2) growing unhealthy (clumped) cells in the same media; (3) spiking the deteriorating culture liquor with several different nutrients; and (4) spiking a healthy outdoor culture liquor with the same set of nutrients. Detailed results of the bioassays can be found in our Interim Report to SERI. To summarize the following conclusions were drawn: (1) iron was not growth-rate limiting in unhealthy indoor pond media, but it was the nutrient that first limited yield; (2) the healthy cells increased themselves 4-10 fold at similar rates in all media and remained 80% dispersed; (3) the unhealthy cells grew as well in all media tested, but remained aggregated; (4) no nutrient deficiency was evident from the nutrient-spiking experiments; (5) initially dispersed cells formed clumps during the nutrient bioassays, with more clumping evident (90% of the biomass) with added $CaCl_2$, but increased clumping (50%) under all treatments. Thus, under low light during short term assays (7 days), both dispersed and clumped cultures grew the same on all media. Furthermore clumping was not found to be reversible by adding any particular nutrient. (Basal medium contained 0.25 mM Ca, 1 mM P, 20 mM alkalinity, and pH was 7.2-7.8.) A low light culture was grown with 0.1 mM P. It clumped as well.

Although several factors may contribute to culture deterioration, including changes in salt balance especially with relation to KCl, NaCl, and $CaCl_2$, photoinhibition appears to be the primary cause. Deterioration was fastest at high light intensity. Even under low light, very dilute cultures exhibited an initial period of pigment bleaching before growth began.

5.4 RECYCLING CLARIFIED EFFLUENTS

Ankistrodesmus 1.4m sq m Ponds Indoors

Ankistrodesmus was grown in the $1.4m^2$ ponds indoors with and without recycle of the medium. The pH was kept at 7.5±.3 and the illumination was diurnal (12:12) at 125 Wm^{-2}. The effluent was clarified by centrifugation (Sharples T1, 20,000 rpm). On average 98% of the effluent was returned to the pond with fresh medium used as make-up. The culture volume was adjusted for evaporation with distilled water prior to removal of the effluent.

Table 5-6 shows productivity from the recycled pond and a control pond, as a function of sequential batch (cycle) number. The productivity is shown graphically in Figure 5-2. As with all of the Ankistrodesmus cultures, productivity from the recycle and control ponds declined slowly with time.

Table 5-6. Ankistrodesmus Media Recycling

	P-1			P-3		
Cycle	Average Prod. $gm/m^2/d$	Maximum Prod. $gm/m^2/d$	Density Range ppm	Average Prod. $gm/m^2/d$	Maximum Prod. $gm/m^2/d$	Density Range ppm
1	20	24	660-710	20	30	160-1300
2	14	20	345-900	16	27	400-1350
3	14	17	350-775	13	15	485-1350
4	17	34	400-1200	11	15	440-1050
5	15	16	450-950	11	17	365-915
6	14	16	385-1360	16	18	350-940
7	14	18	500-1425			
8	11	14	625-1380			
9	14	21	625-1500			

Eventually both cultures became contaminated, the recycle pond somewhat more slowly. Pond characteristics are shown in Table 5-7. Even after eight cycles of recycling, little organic matter accumulated in the medium. Although this experiment lasted only five weeks, a relatively short period of time, it demonstrates that when excretion of organics into the medium is minimal, recycling most of the clarified effluents is feasible. Of course, the organism must be, by far, the most competitive in the given medium, since more practical means of harvesting the biomass will lend a competitive advantage to these organisms which harvest the most poorly.

5.4.2 S. quadricauda 200 sq m Pond Outdoors

An effluent recycle experiment was performed with a mass culture of Scenedesmus for six weeks during June-July 1984. The experiment was terminated when this species failed to grow, in non-recycled ponds as well, during July.

In this experiment, pond effluents were clarified via sedimentation in 2m deep settling ponds. This could be done because of the large size of the flocs (1-2 mm) formed in the growth ponds. Settling ponds were filled by gravity flow from the growth pond. This took about 2 hrs. Sedimentation times varied from 6 to 12 hrs. Clarified supernatents were pumped back to the growth pond using a floating intake. The settled biomass compacted well enough to allow return of about 90% of the efflulent. Fresh medium was

Table 5-7. Media Recycle Dissolved Species

	Start (12-6-83)	Finish (1-13-84)	Units
Alkalinity	20	23	mM
Hardness	400	200	ppm $CaCO_3$
CHO	0	14	ppm Glucose equiv.
Protein	0	14	ppm Folin-Lowry BSA equiv.
TOA	---	---	

used to make up the difference. Irrigation water, without mineral supplementation, was used for evaporative make up water. Urea, phosphate, and iron were added to replenish nutrients taken up by algal biomass production. The operational data, productivity, harvested weight, and efficiencies of harvest are shown in Table 5-8. Clarification efficiency was determined by compositing the effluent recycled, measuring AFDW and dividing by pond AFDW. Slurry density was calculated from the biomass differences between settling pond input and output and the volume left in the settling pond. The culture showed no signs of contamination during the experiment. However, as pointed out above, unless the method of separating cells from the medium is universally efficient, organisms which are moderately competitive but not harvestable would be expected to dominate over the long term.

5.5 CONCLUSIONS

Most of the organisms tested exhibited broad tolerance to changes in total salinity and mineral composition. Although the chemical composition of the medium exerts a strong general selection pressure, it appears that a number of strains can grow well over non-exotic ranges of composition. Thus the major selection forces, in a given general medium, will be those conditions, and changing conditions of climatic inputs and stresses developed under intensive cultivation conditions.

Table 5-8. S. quadricauda Biomass Harvest--Effluent Recycle

200 m^2 Pond

Cycle	Harv. Date	Harv. Eff. %	Sed. Time hrs.	Effl. Dens. ppm	Blowdown %	Evap. loss %	TDS ppm	Hd ppm	Alk mM	Ave. Prod. $gm/m^2/d$	Harv. wt. Kg
0	5/27	99	9	9	12	22	--	--	--	20	25.1
1	6/2	98	8	21	6	16	270	--	--	24.5	24.6
2	6/10	99	12	5	6	25	300	206	7.3	13*	20.3
3	6/17	93	6	55	8	22	326	208	6.6	19	19.6
4	6/24	86	12	87	9	19	450	235	7.5	16.	18.3

* Rotifer bloom, pH raised from 8.3 to 9.3

TDS, Hd, Alk - initial values for succeeding cycle

Hd - ppm as $CaCO_3$

The recycling experiments did not show any deleterious short-term effects like the fast build-up of autoinhibitors. And no unusual problems of contamination were encountered. However, these kinds of questions can only be answered with assurance from much longer scale tests. The contamination problems will be closely tied to the harvesting technique.

SECTION 6.0

MIXING VELOCITY EXPERIMENTS

6.1 INTRODUCTION

Mixing is a critical cost center of any hydraulic system. The provision for some movement of the algal suspension is a necessity in order to take advantage of the cost savings obtained by limiting the number of points of nutrient input and product recovery. Mixing has also been implicated as a determinant of photosynthetic productivity. Thus the major question is how low can mixing power be before productivity is impacted, or more precisely what is the optimum of the mixing power-productivity relationship. In this section the relationship between randomized, turbulent mixing and biomass productivity is discussed. First the methods used to measure channel velocity and power input are presented along with the results from the experimental ponds. Then mixing speed experiments using Scenedesmus and Chlorella are discussed. Finally the results of mixing vs gas transfer through the surface are presented.

6.2 METHODS

The total power required to mix a pond at a given velocity was determined by measuring both the voltage and current to the DC gearmotor (Bodine #185, 1/4 hp, 60:1) which drives the paddle wheel. For a given speed setting, the DC motor controller provides an almost constant voltage to the motor's armature, via its feedback circuitry. The armature current varies directly with motor torque. The product of voltage and current represents the power into the motor, while the product of speed and torque represents the power out of the motor, which is transmitted through the geartrain to the paddle shaft. The load (torque) on the motor, especially at higher mixing speeds, is not constant, but fluctuates six times for each paddle revolution as each of the six paddle blades lifts a new slug of water. The fluctuating current was measured with a chart recorder, which monitored the voltage across the 0.1 ohm resistor placed in series with the motors armature. (The load represented by the resistor was in all cases less than 1% of the motor load.) The average current was later determined from the chart recordings by graphical means. The full armature voltage was measured simultaneously with a Beckman Tech 330 voltmeter, which measures the true RMS value of the voltage. This was important since DC motor controllers put out a non-sinusoidal waveform (especially at low speeds) which are measured incorrectly on non-RMS type meters.

The total power is useful in calculating the overall mixing efficiency, but such an efficiency is strongly dependent on the gearmotor characteristics. Small gearmotors are quite inefficient, so the overall mixing efficiencies calculated will not be representative of what can be achieved on a larger

scale. If the actual paddle wheel shaft power were known, then "paddle efficiencies" could be calculated. Such a number would be a quite useful design tool. Two approaches were taken in estimating the "Net Shaft Power." The first was to simply calculate an efficiency for the gearmotor (motor plus speed reducer) from the manufacturer's nameplate data. Strictly speaking, such a value applies only at full load, and generally declines at partial load. The second approach was to measure the power under "no-load" conditions, i.e., in an empty pond, and to subtract this from the power measured under a load. A comparison of these two methods for calculating net shaft power is shown in Figure 6-1, for paddle wheel speeds ranging from 4.3 to 8.3 rpm, which corresponds to velocities of 15 to 33 cm/sec.

Since the two agree fairly well, and since the former method tends to overestimate net shaft power at lower speeds, the latter approach (using "no-load" power) was used in subsequent calculations. A plot of no-load power vs. paddle wheel speed for the 100 m^2 pond is shown in Figure 6-2.

Velocity measurements were taken with a Nixon Instrumentation Ltd. Steamflow #442 meter equipped with a low speed probe. The probe consists of a very small (~5 mm) propeller which turns on a low-friction spindle. The motion of each propeller blade past a sensor creates an impedance change which is detected electronically. The pulse frequency is averaged over 10 seconds and displayed. Readings were later converted to velocities using the calibration chart suppliled. (The relation is linear for velocities greater than 5 cm/sec.) The measurements were taken at the far end of the pond, right before the first bend, in the center of the channel. It had been found previously that this point was representative of the average velocity in the channel. Readings were made at the bottom, middle, and top of the water column, and generally increased with distance from the bottom as expected in open channel flow. Although some fouling difficulties were encountered, especially at velocities below 15 cm/sec, performance was, for the most part, satisfactory (the instrument is designed for fresh water). Fouling was always accompanied by an abrupt drop in pulse frequency, so it did not occur undetected. In general, the availability of suitable instruments for use at velocities less than 30 cm/sec in shallow water is quite limited.

Given the channel velocity, the total flow rate can be calculated readily. The product of density, flow rate, and head loss is the hydraulic power, or energy per unit time required to keep the water in motion. Given the hydraulic power, total efficiency, paddle efficiency, and equivalent roughness can be calculated. The total head loss is the difference in water levels before and after the paddle wheel. Accurate measurements were difficult, because the levels fluctuate (again six times per revolution), and because the head loss is quite small at low flows. The technique involved clamping rulers to an beam spanning the channel, both before and after the paddle wheel. With the mixing off, the rulers were adjusted to a convenient reference level. The mixing was then turned on, and allowed to come to steady state, at which point the mean deviations from the reference levels were determined visually and recorded. The differences were then averaged to produce a single figure. The paddle wheel RPM was used as a measure of channel velocity, since the two correlate quite well, as shown in Figure 6-3. The minimum velocity for which the total head loss could be confidently measured using this technique was 15 cm/sec (0.4 cm).

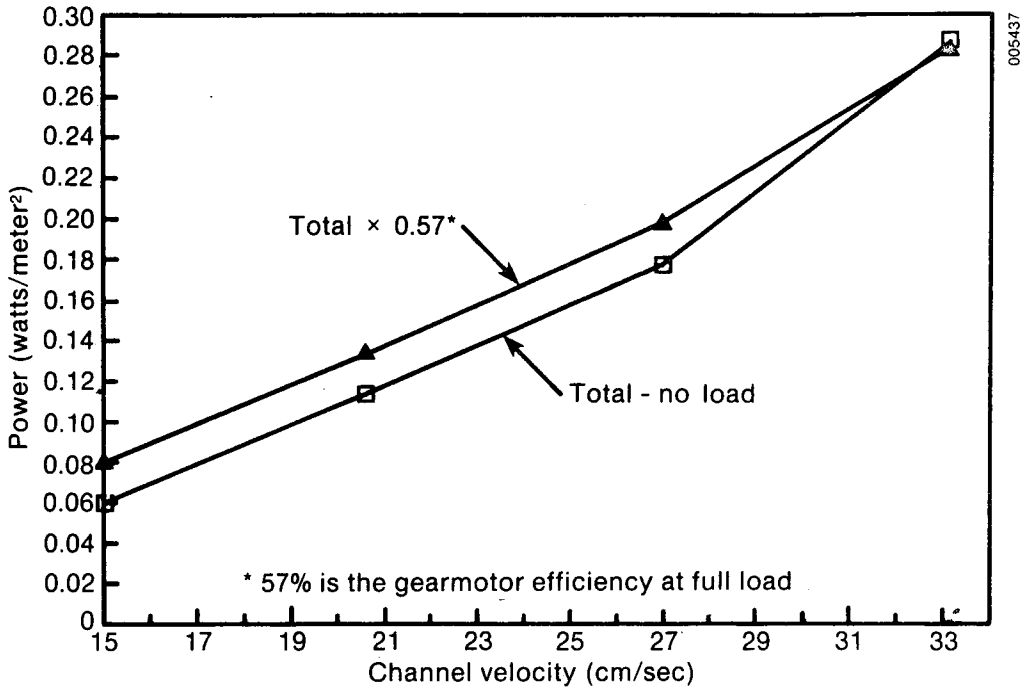

Figure 6-1. Net Shaft Power - 100 m^2 Pond

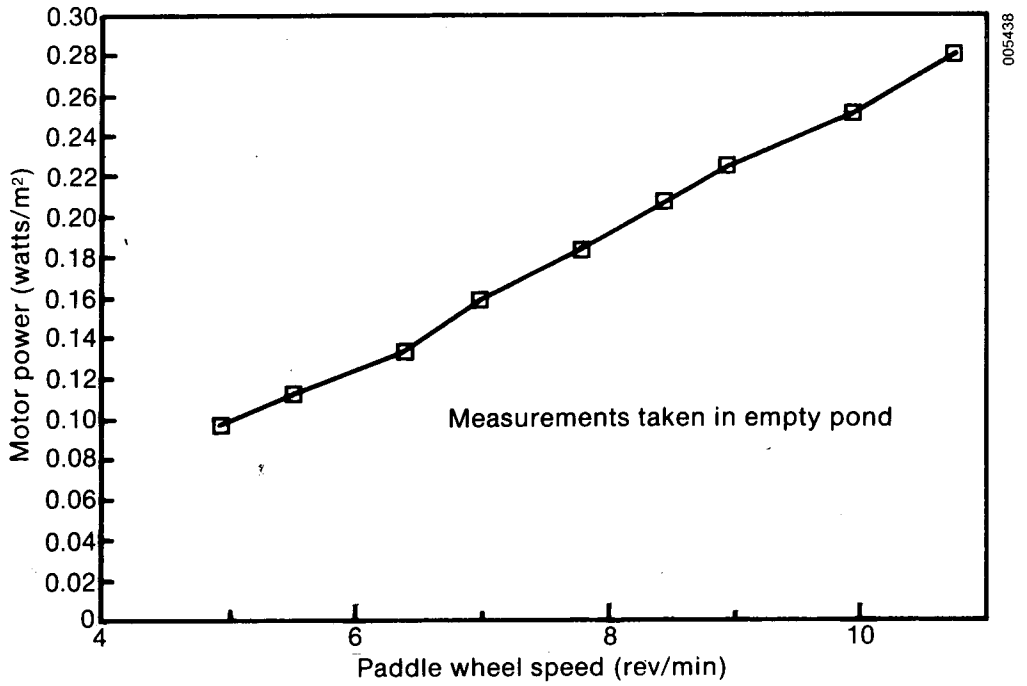

Figure 6-2. No-Load Power - 100 m^2 Pond

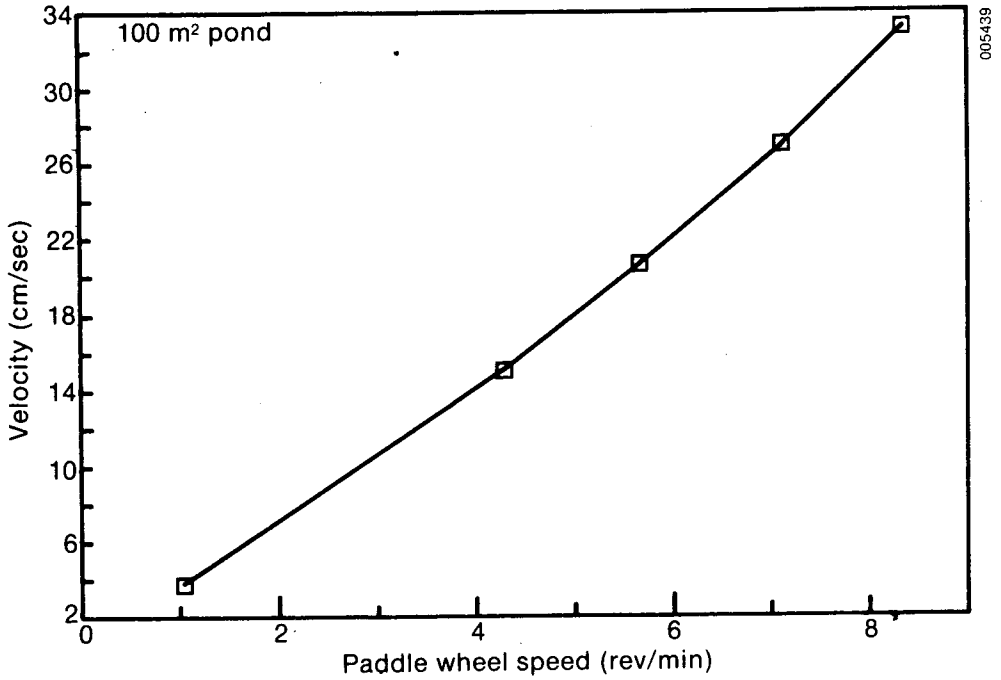

Figure 6-3. Channel Velocity vs Paddle Wheel RPM

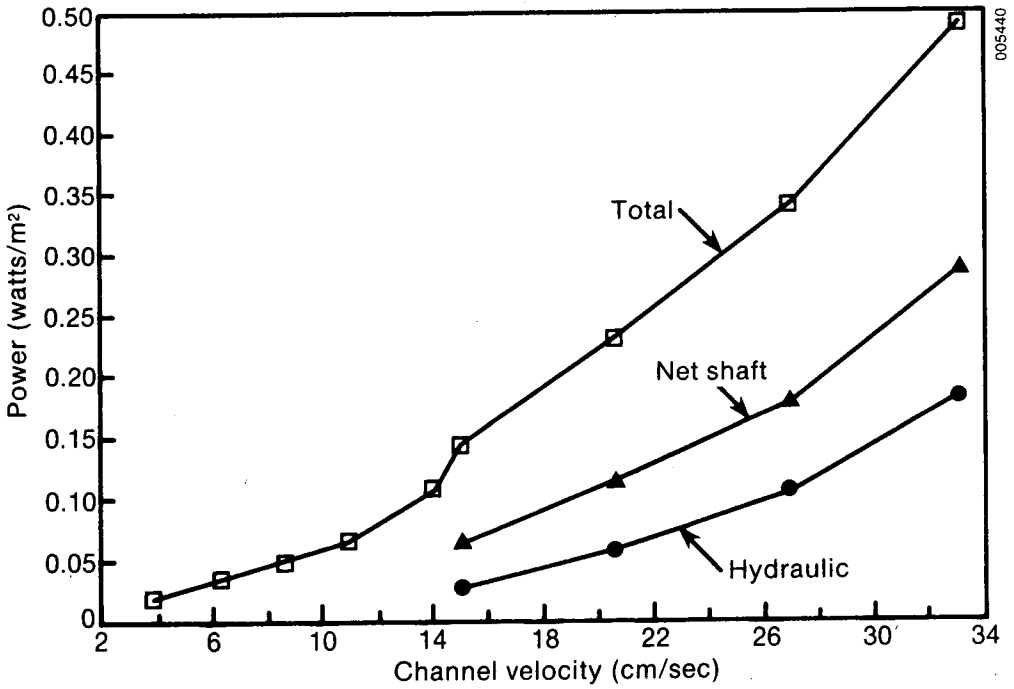

Figure 6-4. Mixing Power - 100 m^2 Pond

6.3 RESULTS AND DISCUSSION

The power and flow measurements for the 100 m^2 pond are summarized in Table 6-1 and shown graphically in Figure 6-4. Total power measurements were made over the motor's entire speed range, corresponding to channel velocities of 3.8 - 33.1 cm/sec. Values below 15 cm/sec were taken at a different time, which may explain the discontinuity between 4.00 and 4.28 rpm. Velocities followed by an "i" are interpolated from Figure 6-2. No-load and net shaft power are only listed for velocities of 15 cm/sec and greater. Below this, the calculated net shaft power approached the probable error in the measurements. Part B of the table lists the values of hydraulic power derived from the velocity and head measurements. It is interesting to note that the hydraulic power varies as the velocity to the 2.5 power (somewhat less than the 3.0 power predicted by open channel flow equations), whereas net shaft power increases roughly as the velocity squared. The discrepancy is due in part to a drop in paddle efficiency at the lower speeds, as shown in part C. The relativelly large paddle clearances (2-3 cm) may account for this decline. The total efficiency dropped even more dramatically at lower velocities, but this is due primarily to the inefficiency of the drivetrain at partial load. At 33 cm/sec the no-load power is 41% of the total power, whereas at 15 cm/sec, it is 57%.

A final quantity that can be derived from the measurements is an equivalent Manning's "n" for open channel flow, which is a measure of channel roughness. Typical values vary from 0.012 for smooth concrete to .029 for gravel. The governing equation is:

$$h_L = L V^2 n^2 R^{-1.33}$$

where: h_L = head loss, meters (Table 6.1)
V = Velocity, m sec^{-1} (Table 6.1)
n = Manning's n, sec $m^{-0.167}$
R = Hydraulic radius = 0.17m (d = 0.2m)
L = Channel length = 45 m

Note: There is no 1.49 factor in SI units.

Two sets of n values calculated from this equation are shown in Table 6-2. In the first, the head losses are used directly, while in the second, they are reduced by a factor of $V^2/2g$ to account for channel bend losses. This value was chosen somewhat arbitrarily, since no value of head loss for sharp bends in open channel flow could be found in available literature. It is consistent with literature values for sharp bends in piping systems. In large scale designs, where channel length to width ratios are high, the bend losses will be a small percentage of the total head loss. However, the 100m^2 ponds have a low L/W ratio of about 8.3/1, and the bend losses probably are a significant fraction. In theory, Manning's n is not a function of velocity, while the calculated values shown in Table 6-2 clearly are. The value shown for 15.0 cm/sec is probably the least accurate, as the uncertainty in the head loss was at its greatest. The values fall within the expected range for a plastic lined pond.

Table 6-1 Mixing Power and Velocity Results

A. POWER MEASUREMENTS

Paddle RPM	Ave Vel cm/sec	DC Volts	DC Amps	Power Watts	Total Power w/sq m	No-load Power w/sq m	+	Net Shaft Power w/sq m
1.07	3.8	11.7	0.16	1.9	0.02		+	
1.79	6.3i	18.5	0.19	3.5	0.04		+	
2.45	8.6i	24.3	0.20	4.9	0.05		+	
3.09	10.9i	30.2	0.22	6.6	0.07		+	
4.00	14.0i	38.2	0.28	10.7	0.11		+	
4.28	15.0	42.0	0.34	14.3	0.14	0.08	+	0.06
5.66	20.6	55.0	0.42	23.1	0.23	0.12	+	0.11
7.10	26.9	68.0	0.50	34.0	0.34	0.16	+	0.18
8.33	33.1	82.0	0.60	49.2	0.49	0.20	+	0.29

i = interpolated value
No-Load Power was measured at the same speed setting in an empty pond.
Net Shaft Power = Total Power - (No-Load Power)

B. FLOW MEASUREMENTS:

	Average			Hydraulic	
Paddle RPM	Head cm	Velocity cm/sec	Flow m /sec	Power watts	Power w/sq m
1.07		3.8	0.0172	0.01e	0.0001e
4.28	0.40	15.0	0.0678	2.64	0.026
5.66	0.64	20.6	0.0931	5.80	0.058
7.10	0.87	26.9	0.1216	10.42	0.104
8.33	1.24	33.1	0.1497	18.26	0.183

Flow is calculated from Velocity x cross-sectional area.
Hydraulic Power is calculated from the measured Head & Flow
e = extrapolated value

C. EFFICIENCIES

Ave Velocity cm/sec	Efficiency Total	Efficiency Paddle
15.0	19%	44%
20.6	25%	51%
26.9	31%	59%
33.1	37%	64%

Total Efficiency = Hydraulic Power / Total Power
Paddle Efficiency = Hydraulic Power / Net Shaft Power

Table 6-2. Roughness Coefficient

Ave Velocity cm/sec	Head cm	Manning's "n"	Adj. Head cm	Adj. Manning's "n"
15.0	0.40	0.019	0.29	0.016
20.6	0.64	0.018	0.42	0.018
26.9	0.87	0.016	0.50	0.016
33.1	1.24	0.015	0.62	0.015

Adjusted Head = Measured Head - $v^2/2g$ (see text)

A single power and velocity measurement was made in one of the 200 m^2 ponds. At 33 cm/sec, the total power was 96 watts, or 0.48 watts/m^2. This is consistent with the value of 0.49 watts/m^2 obtained in the 100 m^2 pond. The corresponding no-load power was not measured.

A final point should be made with regard to the data in this section. While the channel velocities measured span the normal range for practical pond operation, the corresponding head losses all reflect the relatively small scale of the system. Production systems will have much larger L/W ratios, with greater head losses for any given velocity. This situation could be simulated in a small scale pond through the use of obstacles which induce head losses. While such a procedure would invalidate the Manning's roughness calculations, it would be useful in evaluating paddle wheel performance at higher lifts. Any such studies should also incorporate improved paddle wheel design, such as those suggested by Dodd [6]. These include an increase in the number of blades and the offset of adjacent paddle wheel sections to reduce pulsations, and a reduction in backflow through carefully controlled clearances and the use of a depression under the paddle wheel.

6.4 MIXING - PRODUCTIVITY EXPERIMENTS

6.4.1 Scenedesmus Cultures

Two 100m^2 ponds were operated at different mixing speeds during May and early June 1984. The results are given in Table 6-3. No difference in productivity was observed for the range of mixing speed from 15-27 cm/sec. In experiments 1 and 2, the use of turbulence generating obstacles (bricks placed across the channel every eight ft.), which partially organized the mixing into rolling swirls, did not increase productivity. It did, however, greatly increase the power input by increasing the effective Manning's roughness. The lower limit of mixing speeds used in this experiment was 14 cm/sec. When lower speeds were attempted, the clumped biomass settled in the ponds. Thus, about 15 cm/sec appears to be requird to keep flocced material suspended. As experiment 3 shows, randomized mixing regimes at high speed (27 cm/sec) did not increase the productivity of dense cultures either.

Table 6-3. Scenedesmus Mixing Speed Experiments-100m^2 Ponds

Exp't.	Dates	T,°C max/min	pH	Tot. W/m^2	Head cm	Hydr. W/m^2	Prod. $gm/m^2/d$	Days	Vel. cm/sec	TDS
1,I-2	5/6-11	30/14	8.1-8.7	.34	.87	.11	18	6	27	3.9
1,I-1*	"	"	7.8-8.5	.86	---	(.32)	20	6	15	3.9
2.I-2	5/13-26	30/14	8.0-9.4	.34	.87	.11	14	13	27	3.9
2,I-1*	"	"	7.7-8.4	.86	---	(.32)	13	13	14	3.9
3.I-2	5/28-30	33/20	7.8-8.2	.34	.87	.11	18[10]	3[5]	27	1.0
3,I-1	"	"	7.8-8.2	.13	(.40)	(.025)	18[10]	3[5]	14	1.0
4,I-2	6/3-7	25/16	8.3-8.6	.34	.87	.11	16	5	27	0.3
4,I-1	"	"	8.5-8.7	(.18)	(.6)	(.045)	19	5	18	0.3

* Turbulence generating obstacles in pond
( ) Interpolated values

6.4.2 Chlorella Cultures

A more controlled, broader mixing experiment was conducted in August and September 1984 when the Chlorella was being cultivated. This organism lent itself well to mixing experiments since its small size (3-5u) precluded settling, even in practically still water. Thus mixing speeds from <1 to 60 cm/sec were tested. Also, the level of DO, which decreases as mixing speed is increased in this range, was kept constant. This was accomplished by recirculating the suspension at one location in each pond, through a 1/3 hp pump and back into the pond via a two inch PVC pipe across the channel bored with 3/8 inch holes on six inch centers. The water leaving the pipe was directed straight downward to minimize the effect on average mixing speed. The use of this deoxygenating device, wind mixing, and twice a day mixing of all ponds for sampling, explains why the lowest speed used was $\leq$1 cm/sec rather than zero.

Table 6-4 gives all the pertinent data from the experiments. The major result, productivity vs. mixing speed is shown in Figure 6-5. It's clear that for productivity up to 25 gm/m^2/day and mixing speeds from 0 to 30 cm/sec there is no dependence of productivity on mixing speed. This result really is not surprising for slow uncorrelated movement of cells in the water column. Each cell responds to the slowly changing irradiance it is receiving. The turnover of cells even at the lowest mixing speed was apparently sufficient to prevent cell death due either to prolonged exposure to the highest irradiance or to total respiratory decay in the darkest zones at the pond bottom. These cultures grew to maximal densities of about 600 ppm.

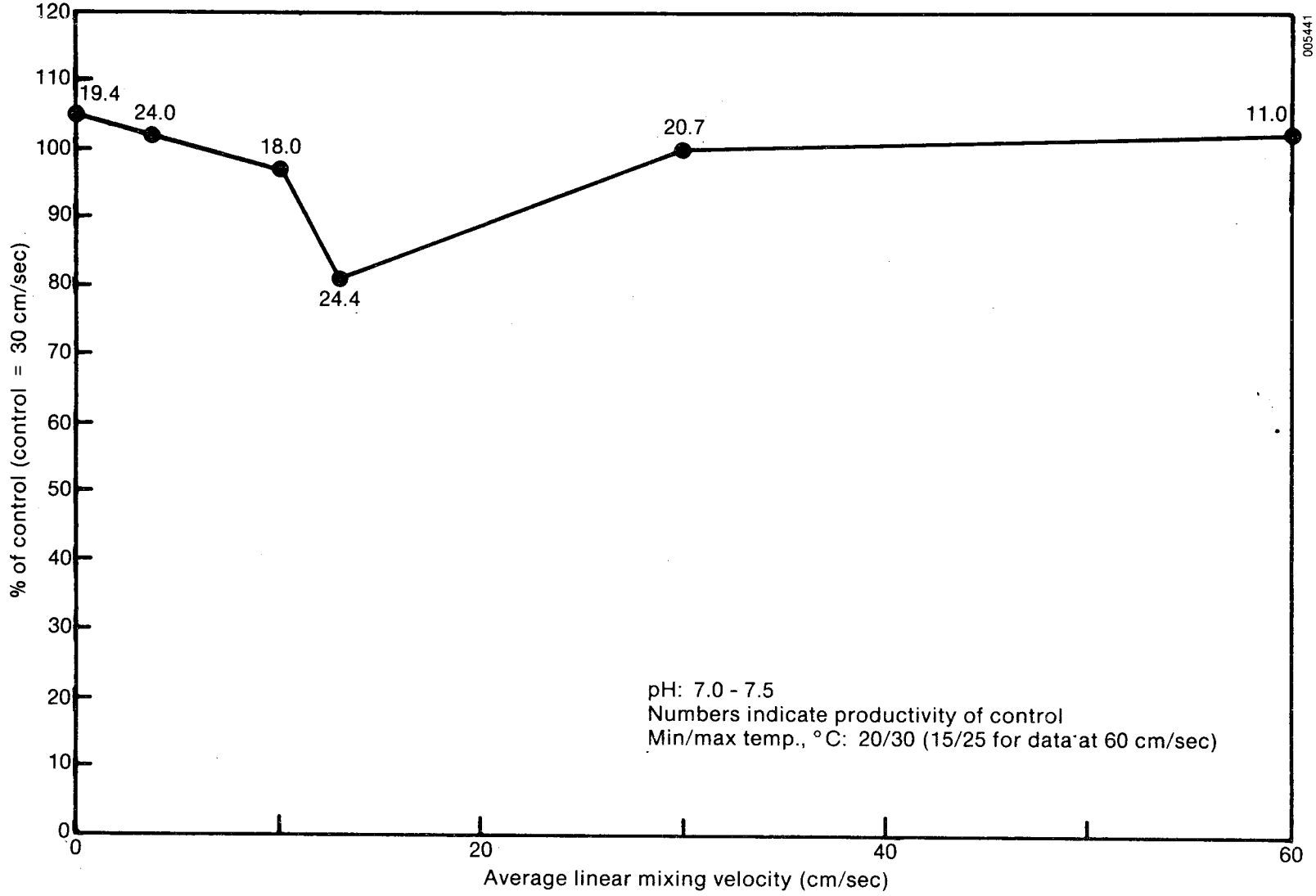

Figure 6-5. Chlorella Mixing Velocity Experiment

Table 6-4. *Chlorella* Mixing Velocity Experiments 100m^2 Ponds

Exp't	Mixing Vel. cm/sec	Hyd. Power Wm^{-2}	Aver. Prod. $gm/m^2/d$	Max. Prod. $gm/m^2/d$	Aver. Insolation Langleys/d	T, °C min-max	Duration Days
1	30	.18	19.1	27.5	496	19-28	5
	< 1	~0	20.0	25.1	496	74-594	5
2	30	.18	23.6	25.3	554	30-580	5
	3.8	.001	24.2	27.0	554	34-639	5
3	30	.18	17.7	23.6	601	69-316	3
	10	.01	17.1	22.5	601	80-337	3
4	30	.18	24.0	34.4	610	75-410	3
	13	.02	19.5	28.0	610	75-368	3
5	30	.18	21.0	-	525	99-587	5
	30	.18	20.2	-	525	117-622	5
6	30	.18	19.7	-	488	54-328	3
	30	.18	20.5	-	488	73-380	3
7	33	.18	10.2	-	491	71-214	3
	60	1.02	10.7	-	491	81-242	3
8	33	.18	11.5	14.6	427	40-254	4
	60	1.02	11.3	15.3	427	52-278	4

Maximum DO at 30 cm/sec 220% sat., 14.5ppm
at 3.7cm/sec 285% sat., 19.0ppm

The low productivities at 60 cm/sec were due to rather abrupt decrease in pond temperatures which occurred in late Sept. and early Oct. 1984.

6.5 MASS TRANSFER THROUGH THE SURFACE

The mass transfer coefficient, K_L, was measured at two mixing velocities: 15 and 30 cm/sec. The measurements were done by measuring the rate of decrease of total carbon from a 100 m^2 pond with known alkalinity. The carbon level was calculated from the carbonate equilibrium equations with constants adjusted for temperature and ionic strength. pH was measured using a Radiometer pH meter capable of accurately measuring .01 pH units. The pH measurements were monotonically decreasing. The range of pH, 7.4 to 7.2, was chosen to preclude effects from back pressure. In the given system, the

driving force of CO_2 in the bulk liquid was about 0.61 mmolar. Air equilibrates to about 0.01 mmolar. The resistance coefficient was calculated as:

K_L = transfer rate /(610-10): meters per hour

for 30 cm/sec,, K_L = 0.1 m/hr. For 15 cm/sec, K_L = 0.038 m/hr.

Calculations for the mass transfer coefficients under pond conditions were made by Weissman [7], using empirical correlations derived from channel and stream aeration studies [8]. The results yielded an equation of the following form:

$$K_L = 0.0169\ (SV)^{0.375}$$

$$K_L = CV^{1.125}/d^{0.5}$$

S = hydraulic slope

V = mixing speed, ft/sec

d = depth, ft.

Substitution of the relevant numbers yields the following values for K_L at 30 and 15 cm/sec respectively: 0.027 and 0.0124. Although the theoretical values are high by a factor of $3.4 \pm .3$ the relative ratio is off only by 20%. Assuming this relationship to be true, Table 6-5 gives outgassing coefficients as a function of mixing velocity. It can be readily appreciated that high mixing velocities (or equivalently high turnover rates of bulk liquid to the surface) will result in very high outgassing coefficients. The relationship between mixing and CO_2 loss will be explored in the next section in which the expected levels of dissolved CO_2 are discussed.

6.6 SUMMARY

Channel velocity and mixing power measurements were made over the range of 3.8 to 33 cm/sec. A Manning's roughness of about 0.016 was calculated for the large, plastic-lined ponds. The overall efficiency of the paddlewheel system varied with speed, being almost 40% at 33.1 cm/sec and 20% at 15 cm/sec. 40-50% efficiency is considered close to the maximum practically achievable.

The dependence of hydraulic power on channel velocity was just less than cubic, basically confirming the steep increase found in "ideal" open channel flow.

Table 6-5. Outgassing Coefficient vs Mixing Velocity (d = 20 cm)

K_L, m/hr	V, cm/sec
.012	5
.026	10
.040	15
.063	20
.10	30
.22	60

The production of biomass was found not to depend on channel velocity over a large range of the latter. Thus other considerations, like carbonation and particle suspension will determine mixing speed. The cost of mixing is very low at low mixing speed, but increases quickly. At 15 cm/sec, mixing power required (at 100% efficiency) was 0.026 Wm^{-2}. At 24 hr/day mixing and 20 gm/m^2/day algal productivity, this is equal to about 0.03 kw.hr/kg algae or about $.003/kg algae produced. On an energy basis the mixing power required represents only 0.5% of the heat of combustion of the algae output. At 30 cm/sec these numbers changed to $.02/kg algae and 3.3%.

SECTION 7.0

CARBONATION

7.1 INTRODUCTION

Carbon dioxide input is another critical cost center of algal biomass production. The cost is determined by the actual cost of CO_2, the capital cost of distribution and injection, and the efficiency of its use. The subjects of this section are the factors which determine this efficiency. They include injection efficiency, outgassing loss, and the physiological response of the organism to pCO_2, pH, and alkalinity.

7.2 INJECTION EFFICIENCY

Injection losses were based on three experiments detailed in Table 7-1. The procedure used included carbonating the ponds and recording initial and final pH, temperature, and alkalinity. After calculating the difference in total inorganic carbon, the outgassing loss, based on mass transfer coefficients measured previously was subtracted. The experiments were performed in irrigation water with alkalinity added, attaining the total alkalinity specified in the table. Injection efficiencies of 57%, 57%, and 66% were measured. For subsequent calculations the value of 66% was used since conditions most closely resembled the ones which yielded this result. The injection efficiencies obtained are much higher than one would normally expect sparging into a water column of only 20 cm. The mass transfer across a bubble surface is very sensitive to bubble size. Although an equilibrium size of 1 cm is usually attained, it takes about 1 m of rise for equilibrium to be reached. Thus shallow sumps are more efficient, per meter of depth than deeper ones.

Table 7-1. CO_2 Losses at Injection

Pond Size m^2	T ^oC	Alk. mM	pH_i	pH_f	CT_f-CT_i Moles	Inflow Rate LPM	Cin* moles	Effic. %
200	29	6.6	9.5±.1	8.5±.05	31.6	8	55.3	57
100	20	5.4	7.78±.01	7.28±.01	0.3	15	4.1	66
100	20	5.4	7.38±.01	7.28±.01	2.8	15	5.0	57

*Corrected for outgassing

7.3 CO_2 UTILIZATION EFFICIENCY

The overall CO_2 utilization efficiency was measured by two methods. In the first method, the biomass production from 2 X 100m^2 plus 1 X 200 m^2 was totalled over 42 days of operation (5/8-6/20) and then divided by the total CO_2 used, as measured by the difference in the CO_2 meter readings on the six ton CO_2 tank (Table 7-2). The 3.3 kg CO_2/kg AFDW corresponds to a 30% efficiency, or 54% of that theoretically achievable. This includes injection losses and outgassing losses, but does not account for urea-derived CO_2. The latter only contributes 5% of the carbon input and thus could maximally reduce the efficiency to 29% overall or 52% of theoretical.

Table 7-2. Overall Carbon Dioxide Utilization Efficiency
5/8-6/20, 1984

1.	Algal biomass produced in 400 m^2 of ponds:	242 kg AFDW
2.	CO_2 level drop in six ton tank	797 kg CO_2
3.	Urea carbon: (242/10/0.46) X 12/60 = 10.5 Equivalent CO_2: 10.5 X 44/12	39 kg C_2
4.	(Algal biomass/CO_2 input) X 100	29%
5	Theoretical 1/1.8	55.6%
6.	% Theoretical attained	52%
7.	CO_2 Requirement: 797/242	3.3 kg/kg

In the second method, CO_2 meters and timers, which were installed on the 100 m^2 ponds in August 1984, were used to tally the total CO_2 input to the ponds. The equivalent algal biomass that the total daily CO_2 input could have supported (based on 1.8 kg CO_2/kg algae) was calculated. The actual productivity was divided by this number. The results are shown in Tables 7-3,-4. Also shown in these tables are the losses used for injection and calculated for outgassing under the stated conditions.

There was substantial variability in the overall efficiency (SD = 10-25% of mean values). Thus the total accounting of CO_2 (into algae, lost upon injection, and outgassed) is only very approximate. The injection loss was large, but this should be reduced easily to 5% or so by proper design of a carbonation station. Not unexpectedly, overall efficiencies were greatest at lower mixing speeds where outgassing is reduced. Thus the interplay between algal productivity, mixing velocity, and dissolved CO_2 concentration will determine the economics of CO_2 utilization.

Table 7-3. CO_2 Partitioning $100m^2$ Ponds

Mixing cm/sec	Aver. $gm/m^2/d$	CO_2 in $gm/m^2/d$	Overall Effic. %	Inj. Loss %	Outgassing Loss %	Tot. %
30	22.9	49.8	48.4	34	20.7	103.1
n = 7	± 5.4	±12.9	±13.7			
30	15.6	36.3	43.0	34	27	104
n = 3	± 3.1	± 6.3	± 3.6			
13	25.2	41.6	64.7	34	5.5	104.2
n = 2	± 3.9	± 3.2	± 4.2			

pH = 7.5 Alk = 5 mM
K_L = 0.1 m/hr for 30 cm/sec, 0.038 for 13 cm/sec

Table 7-4. CO_2 Partitioning with Oxygen Desorber

Mixing cm/sec	Aver. $gm/m^2/d$	CO_2 in $gm/m^2/d$	Overall Effic. %	Inj. Loss %	Outgassing Loss %	Tot. %
30	18.9	42.9	43.8	34	22.4	100.2
n = 5	± 4.9	± 4.9	±10.8			
3.7	24.2	40.4	60.3	34	1	95.3
n = 3	± 1.6	± 4.6	± 8.1			

pH = 7..5 Alk = 5 mM
K_L = 0.1 m/hr for 30 cm/sec, 0.02 for 3.7 cm/sec

An interesting feature of the data is that the mechanism used for desorbing oxygen had apparently little effect on carbon mass transfer. This, too, was expected, since it was obseved that the oxygen desorbed was mainly from nucleated release of supersaturated O_2 and not mass transfer.
Measurements of DO before and after the operation of the desorber for 10 minutes showed that DO decreased 15%, but pH measurements showed no decrease.

7.4 PHYSIOLOGICAL ASPECTS OF CO_2 UTILIZATION

7.4.1 Laboratory Results

Since outgassing of CO_2 is so dependent on dissolved CO_2 levels, and thus can be very high or very low, experiments were conducted to investigate the growth response of the Chlorella as a function of dissolved CO_2, pH, and alkalinity. Since alkalinity is usually a given, the pH of operation will determine the CO_2 level. But experiments with differing alkalinities were required to yield specific CO_2 levels independent of pH. Fortunately this organism exhibited a very broad range of tolerance to alkalinity, as discussed below.

The 1L Roux bottle apparatus was used in these experiments. Temperature was 30 ± 1^0C. Illumination was continuous at 40 Wm^{-2} but only from one side of the bottle. Gas transfer rates were measured and made sufficient to keep dissolved CO_2 essentially in equilibrium with the inflowing gas phase for $pCO_2 \geq 0.025$. That is CO_2 input was an order of magnitude greater than maximal uptake at these pCO_2 levels. For experiments in which air was used, even at 5 LPM with several bubblers, input could not keep up with demand. Of course this was observed as an increase in measured pH. The actual concentration of dissolved CO_2 was estimated by input minus demand. The demand of the cultures was quite constant over a 3 to 4 day period. Only at the beginning, when cultures were dilute, was input much greater than demand. Thus maximum specific growth rate results and average production results obtained from the same culture are referenced to different CO_2 levels, e.g. .033% and .01% respectively.

The results of over 30 experimental runs are shown in Tables 7-5, 6, and Figures 7-1, 2. Reproducibility was quite good. Neither maximum specific growth rate nor average production depended on alkalinity. Both were dependent on pCO_2 and pH. However, production was more sensitive than u_{max}.

Average production was more dependent on CO_2 level than pH. At 2% CO_2, pH hardly mattered between 7.0 and 9.0. At 0.5% CO_2 there is some dropoff at pH >9.0. There is no significant difference between productivity at 0.5% and 2%. The lowest CO_2, 0.01% (one third air equilibrium) resulted in the lowest production at all pH, but still showed (as in the case of 0.5% CO_2) a 25% decrease at pH >9 relation to pH 7. An important conclusion is that pH cannot be used as a variable independent of pCO_2, unless care is taken to vary the two independently. This is not usually done in "pH optima" experiments. In this experiment the effect of pCO_2 on productivity is seen to be more pronounced than that of pH.

The same was found to be true of u_{max}, but to an even greater extent. There was no correlation between u_m and pH, so all of the data is grouped by pCO_2 only. Even the dependence on CO_2 level is less pronounced, with only a 25% reduction in u_{max} compared to a 40% reduction in productivity as pCO_2 is decreased. Maximum specific growth rate determinations are not good predictors of productivity.

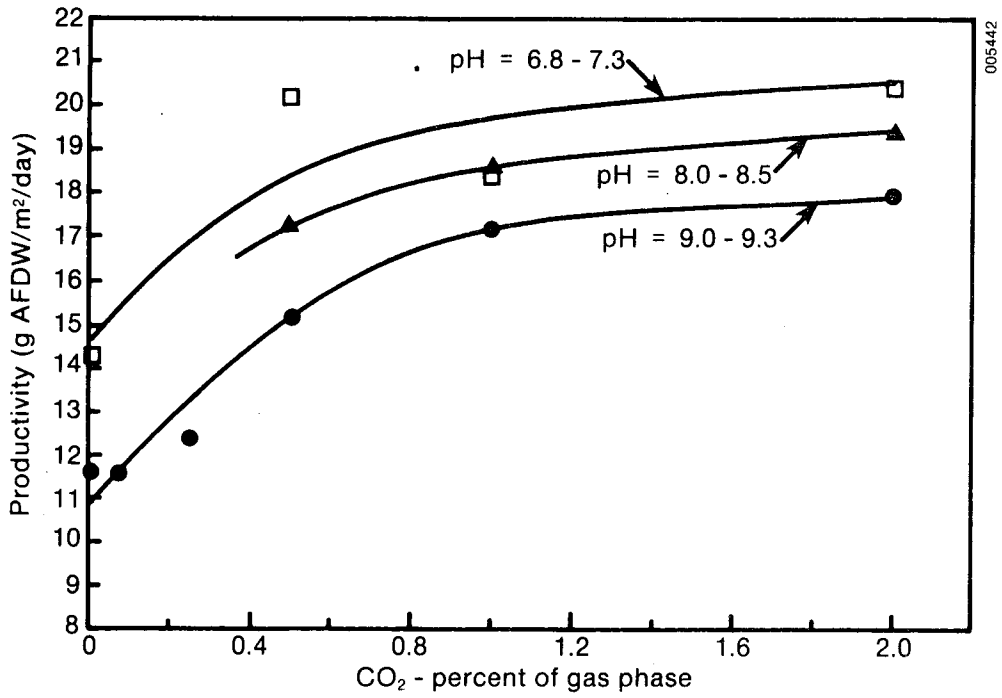

Figure 7-1. Chlorella: Productivity vs pCO_2, pH

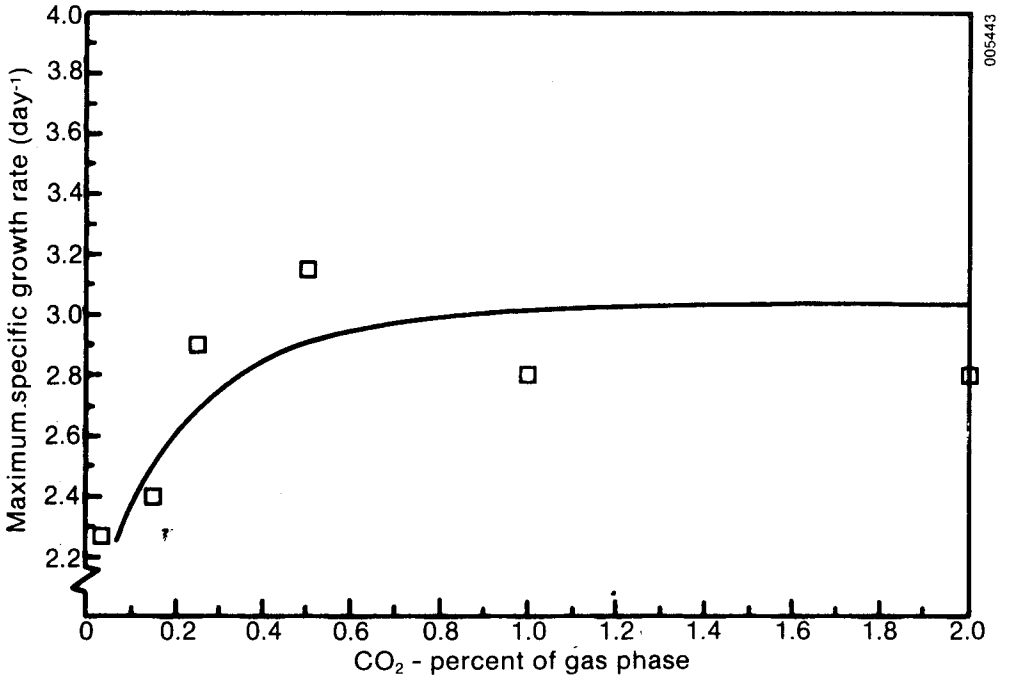

Figure 7-2. Chlorella: Maximum Specific Growth Rate vs pCO_2

Table 7-5. *Chlorella* Productivity vs pCO_2, pH, Alk.

pCO_2 %	pH	Aver. Prod. $gm/m^2/day$	S.D.	Alk. mM	n
2	7.0-9.0	19.1	2.1	2-150	9
2	7.0-7.3	20.4	3.3	2-5.3	3
2	8.0-8.5	19.4	0.4	5.25	2
2	9.0	17.9	1.1	100-150	4
1	7-9.3	18.0	0.7	5-100	5
1	7.6	18.4	-	5	1
1	8.35	18.6	0.2	10	2
1	9.3	17.2	0.0	100	2
0.5	6.8-9.3	18.2	2.9	0.1-50	9
0.5	6.8-7.4	20.2	2.1	0.1-8.0	5
0.5	8.5	17.3	-	20	1
0.5	9.0	15.1	0.5	20-50	3
0.5	9.35	14.6	-	25	1
0.25	9.35	12.4	-	60	1
0.08	9.35	11.6	-	5	1
0.01	7.0-10.35	12.9	1.8	0.1-60	8
0.01	7.0-7.3	14.3	1.6	0.1-1	3
0.01	9.35	11.6	1.7	5	3
0.01	9.8-10.35	12.8	0.0	5,60	2

Table 7-6. *Chlorella* Maximum Specific Growth Rate vs pCO_2, pH, Alk.

pCO_2 %	pH	μ_{max} d^{-1}	S.D.	Alk. mM	n
1,2	7.0-9.31	2.80	0.22	1-100	4
1-2	9.38	2.5	-	100	1
0.5	6.8-9.35	3.15	0.13	.1-60	4
0.25	9.35	2.90	-	5	1
0.15	9.7	2.4	-	10	1
0.033	7.1-10.35	2.27	0.58	1-60	3

7.4.2 Outdoor 1.4 Sq. Meter Pond Results

Some CO_2, pH experiments were performed in the 1.4 m^2 ponds outdoors using the Chlorella (Table 7-7). Productivity was equal to normally operated cultures (pH 7.5, Alk = 5 mM) as long as dissolved CO_2 was kept high enough. In experiment #1, CO_2 was kept low by controlling pH at 8.3 and 9.0. Average production was reduced about 25-30%. Low pH combined with low alkalinity, or high pH and high alkalinity (#2) yielded essentially equal productivity. In both cases dissolved CO_2 was high (equivalent to equilibrium with a 1-2% CO_2 gas phase).

Table 7-7. pCO_2 and Chlorella Productivity: 1.4m^2 Ponds

#	Days	T, °C min-max	pCO_2 mM	pH	Alk. mM	Aver. Prod. gm/m^2/day	Max. Prod. gm/m^2/day
1	5	14-28	0.04	8.3	5	16	30
	6	15-30	0.007	9.0	5	15	-
2	5	15-28	0.75	8.3	100	22	31
	5	15-28	0.3	7.5	5	24	31
3	4	12-25	1.5	7.5	20	21	24
	4	12-25	1.5	7.5	20	20	23

7.5 CO_2 OUTGASSING

Once the response of an alga to CO_2 level and pH is known (or better yet to cycles of changing CO_2 and pH) the specific economics of the use of that strain can be determined. Choice of an organism will depend on the trade-off between productivity at increased CO_2 level and CO_2 loss due to increased outgassing. As an example, the equivalent algal biomass production potential of outgassed CO_2 is plotted in Figures 7-3 and 7-4 as a function of pH and alkalinity (that is, dissolved CO_2 concentration) for two different types of waters. The first is similar to the medium used for growth of Chlorella in this project. The second is a hypothetical water with the same equilibrium constants (for the carbonate system) as seawater. It isn't seawater since the addition of the indicated amounts of alkalinity would result in $CaCO_3$ precipitation from seawater. It is simply a high TDS case.

If a given medium, after evaporative concentration contains, for example, 20 mM alkalinity then the range of dissolved CO_2 (pH) at which outgassing is minimal is restricted. The best approach is to screen for organisms which are highly productive within this acceptable CO_2 range. If productivity increases as CO_2 level increases, the increase must justify the larger CO_2 outgassing loss. At about 20 mM alkalinity losses are not too severe in low TDS waters down to pH 8 or so, in high TDS waters down to pH ~7.7.

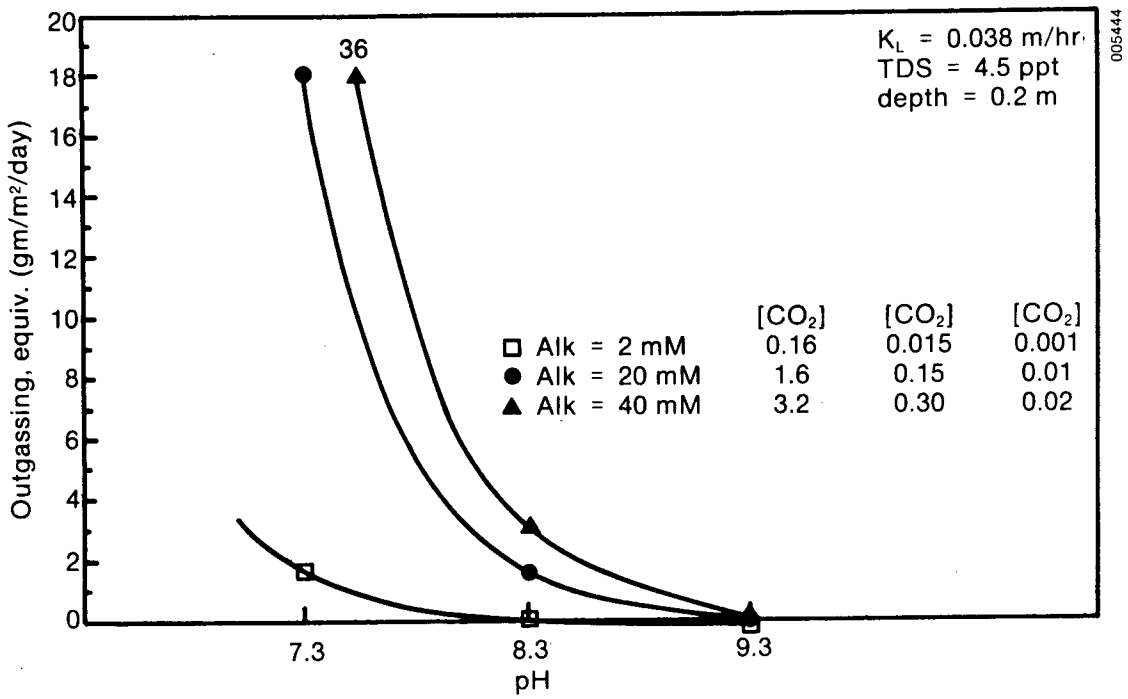

Figure 7-3. CO_2 Outgassing - 4.5 ppt TDS

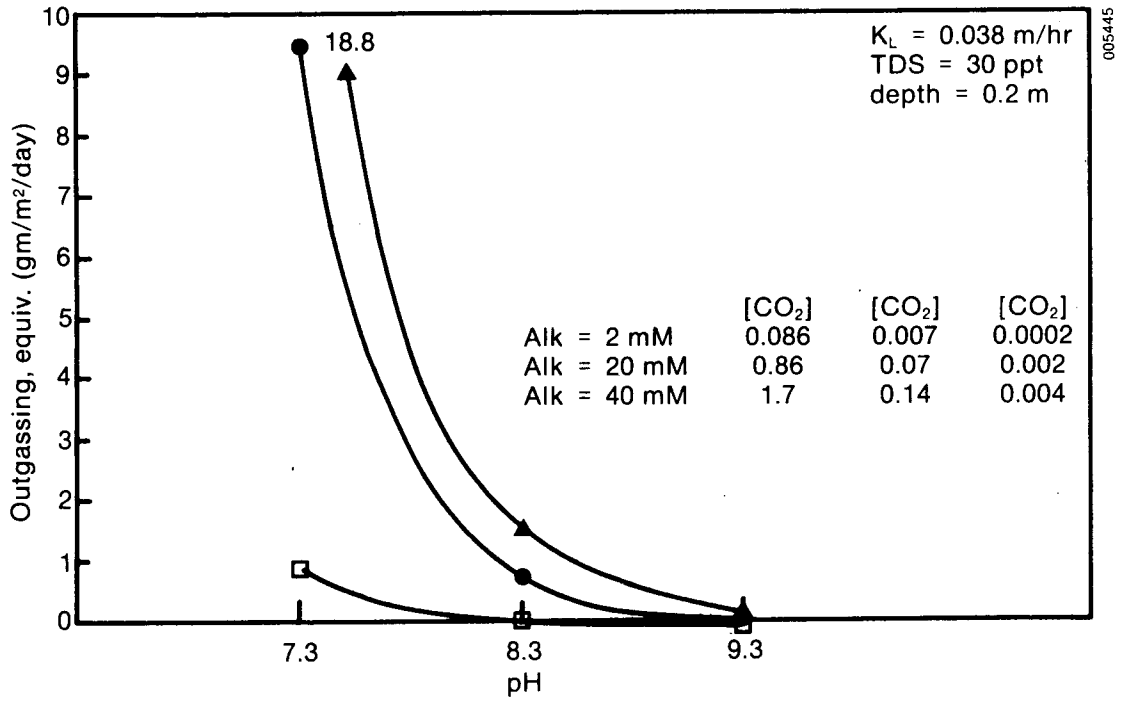

Figure 7-4. CO_2 Outgassing - 30 ppt TDS

The corresponding dissolved CO_2 concentration is about .3 mM (equivalent to equilibrium with a 1% CO_2 gas phase) which does not limit productivity of the Chlorella. However, pH would rise in between carbonation stations, lowering CO_2 to .07 mM (at pH 9.3 or 8.7) which might impact productivity. However, operating closer to pH 7 becomes uneconomical unless alkalinity is very low as in a seawater system without recycle. Even so, the lack of alkalinity lowers the carbon storage capacity so much that the distribution system for CO_2 injection would become prohibitively expensive.

The above discussion is based on a mixing speed of 15 cm/sec. If this is increased to 30 cm/sec, then operation below pH 8 becomes expensive, since outgassing increases 2.5 fold. If the pond is operated at 10 cm instead of 20 cm, the outgassing is increased another factor of about 1.5. In addition, any mechanism which increases the efficiency of vertical movement of water will further increase outgassing losses.

7.6 CONCLUSIONS

The major losses of Co_2 were incurred upon injection and due to outgassing through the pond surface. The former is easily reduced to below 5% by sparging into the bottom of a one meter deep sump. The latter can only be reduced by operating at lower concentrations of dissolved CO_2, by lowering channel mixing velocity, and by increasing pond depth. Thus an optimization problem arises, particularly with respect to biomass production as a function of CO_2 concentration. Since alkalinity can be assumed to be moderate to high (required for adequate carbon storage and a given in most groundwaters after evaporative concentration), pH must also be moderate to high. The growth characteristics of the organism in terms of CO_2 must be known in order to evaluate the economics of the system. The usual pH optimum experiments are not satisfactory. In the final analysis, the strain screening protocol should include the type of experiment described above. Bicarbonate uptake, which was not significant in the work reported here, should also be measured since it may significantly increase productivity at low CO_2 (high pH).

SECTION 8.0

STORAGE PRODUCT INDUCTION

8.1 OUTDOOR CULTURES

In order to induce accumulation of storage products, cultures were grown past their nitrogen-sufficient growth potential. Effluents from continuously operated ponds of both S. quadricauda and S. SO2a were diluted with nitrogen free medium and allowed to "induce" in batch. Batch cultures of both Scenedesmus strains and the Ankistrodesmus were grown on yield-limiting nitrogen levels. In general none of the organisms tested increased in lipid content after nitrogen-starved growth. Total carbohydrate content did increase. The S. quadricauda strain was productive after nitrogen depletion and after readdition of nitrogen. The S. SO2a grew after nitrogen depletion but productivity declined compared to N-sufficient growth. The Ankistrodesmus grew poorly when N-starved and did not recover; it did not resynthesize pigment and eventually died, after readdition of urea-nitrogen.

8.1.1 Induction of Continuous Pond Effluents

Effluents from continuously grown cultures of S. quadricauda were used for growth, in batch, on N-deficient media. Table 8-1 shows total, lipid, and carbohydrate productivities of those effluents that actually became nitrogen depleted. The effluents were diluted with nitrogen-free medium to lower initial nitrogen levels. The time course of one of the runs is shown in Figure 8-1.

In general, productivities of nitrogen-starved cultures were as high as non-starved batch cultaures operated at the same time. This was especially true if starvation did not proceed past 2-3 days. Severe drop offs in productivity occurred thereafter. There often was a day of lowered productivity as nitrogen depletion set in, although variable insolation make this uncertain. Carbohydrate content doubled and lipid content dropped 25%.

8.1.2 Batch Growth and Batch Induction

Batch cultures of three strains were started with minimal levels of urea to result in depletion of nitrogen from the medium. For two-three days after N depletion induced cultures of S. quadricauda were as productive as uninduced cultures (Table 8-2). Again, lipid content dropped 25%. Carbohydrate content was not routinely measured in these experiments. The cells used in runs 9-11 were N-deficient before addition of 10 ppm urea-N at the start of each run. As well, during Run #10 100 ppm of urea-N was added after day 4. The culture increased in productivity (to 16 gm/m^2/day) over the next day and a half. These results indicate that this strain recovers quickly from N-starvation. A typical time course of induction is shown in Figure 8-2.

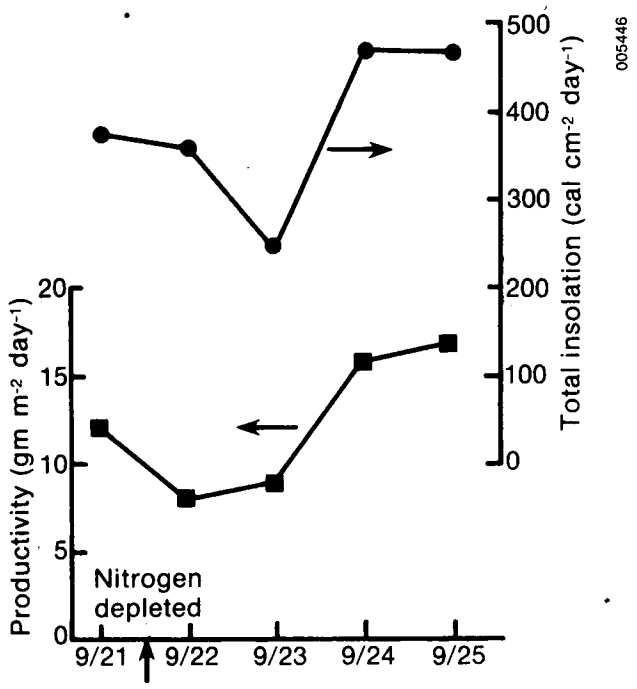

Figure 8-2. Batch Induction of Continuous Pond Effluent

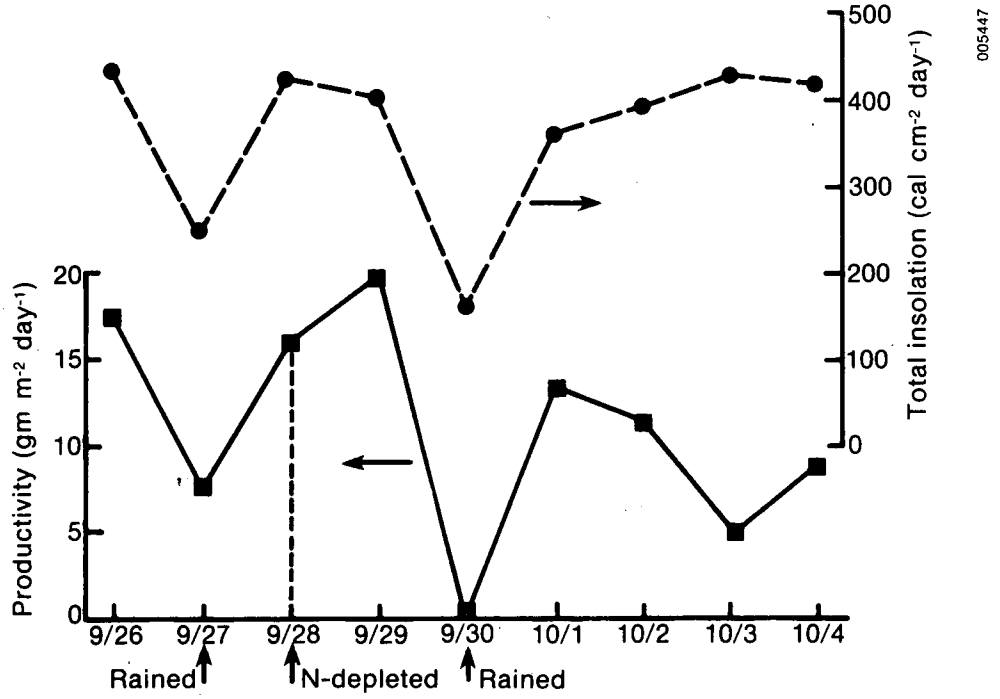

Figure 8-2 Nitrogen Depleted Batch Growth

Table 8-1. Batch Induction of Continuous Pond Effluents

Date	Day	% Lipid	Lipid Prod.	% CHO	CHO Prod.	Total Prod.	Lqly/day total
S. quadricauda							
9/11	0	26.6	3.5	25	3.5	14.0	530
9/12 --	1	-	-	-	-	7.4	514
9/13	2	16.3	(-.4)	42	(6.6)	10(8.7)	531
9/14	3	14.7	-.3	47	4.3	3.4	522
9/15	4	14.0	-.5	55	4.0	0	514
9/21 --	0	20	2.5	25[1]	3.2	12.6	376
9/22	1	-	-	45	9.0	8.2	361
9/23	2	-	-	-	-	9.0	249
9/24	3	14.2	(3.2)	-	-	15.3	474
9/25	4	-	-	-	-	15.8	473
S. SO2a							
10/7	0	19.5	0	25	-	8.2	295
10/8 --	1	15.9	0.6	42	12	14.0	230
10/9	2	15.9	0.2	51	5	10	40

[1]Initial values at beginning of batch growth.
( ) indicate an average value. Productivities in $gm/m^2/d$
-- Indicate depletion of N from medium.

Table 8-2. *S. quadricauda* Batch Induction

Run #	Prod. Before N-depletion $gm/m^2/day$ Max.	Avg. (Days)	Prod. After N-depletion $gm/m^2/day$ Max.	Avg. (Days)	Avg. (Days)
9	17.6	13(2)	19.7	18(2)	12(5)
10	18.2	13(2)	18.0	13(2)	--
11	17.3	13(2)	17.6	15(3)	9(5)

The nitrogen-starved cultures of *S. SO2a* increased from 25% to 60% carbohydrate content, but productivity was lowered. The *Ankistrodesmus* grew poorly after depletion of N from the medium (7 $gm/m^2/day$) and carbohydrate content only increased to 32%.

8.2 INDOOR CULTURES

Tests for storage product induction were performed in the lab with I. galbana, Ankistrodesmus, and S. quadricauda, to determine the potential for increases in fuel precursors under more easily controlled conditions. Batch cultures were grown in 1L Roux flasks, under continuous illumination of 25 wm^{-2} at pH 7.5-.5 and T=28±2°C. The results are shown in Table 8-3, along with some data previously taken by the author for comparison.

Table 8-3. Storage Product Accumulation in Laboratory Cultures

	Productivity gm/m^2/day				Storage Product			
ALGA	N-Suffic. Avg.	N-Suffic. Max.	N-Starved Avg.	N-Starved Max.	Type	Max. Cont. % VSS	Max Prod. gm/m^2/d	Ave Prod. gm/m^2/d
Ankist.	10	14	8	10	CHO	50	--	4
S. quad.	10	14	8	10	CHO	60	8	5
S. acutus	10	15	12	20	CHO	75	15	9
S. platensis	9	10	11	15	CHO	80	15	9
D. tert. 999								
Seawater	11	17	13	20	CHO	75	--	--
2.4 M NaCl	6	8	--	--	CHO	--	--	--
I. galbana								
Hi density	7	10	7	9	Lipid	40	4	3
Lo density	7	10	1	--	Lipid	63	--	0.6

SECTION 9.0

BIOMASS HARVESTING

9.1 INTRODUCTION

Harvesting microalgal biomass from dilute suspension has been one of the most persistent problems in the development of production technology. It is particularly difficult when the size of the organism is small and the value of the biomass is low. In this section results from sedimentation tests are presented. The S. quadricauda, due to its agglomeration into large clumps, was unusually easy to harvest via sedimentation without flocculants. However, data is presented which indicates that nitrogen-starved single cells sediment more rapidly than nitrogen-sufficient cells. Thus the sedimentation of nitrogen-starved biomass may be feasible.

9.2 SEDIMENTATION RATES - LABORATORY RESULTS

Samples from the outdoor ponds were taken to the laboratory to measure biomass sedimentation rates. The rates of clarification were based on turbidometric assays. The tests performed were not aided by chemically induced flocculation. Flocculants can lead to greatly increased rates of clarification, but must be evaluated in terms of cost per unit biomass concentrated. Also, the effects of residual flocculants in the media, which must be recycled many times, are unknown.

The data from sedimentation tests are summarized in Table 9-1. It is clear that the naturally flocculant culture, S. quadricauda, grown in batch, sedimented much more quickly and completely than any other culture. Nitrogen starvation of this clumped culture did not have much effect on clarification rates. When this organism was grown in continuous culture, it did not aggregate. The sedimentation rates reflect this with only 40-60% of the biomass settling faster than 15 cm/sec. Effluents from continuous ponds, "induced" in batch, settled somewhat faster than uninduced biomass with 65-75% sedimenting faster than 15 cm/sec. Sedimentation of Ankistrodesmus and S. SO2a cells was not very rapid. Again, induction (nitrogen starvation) increased the rates.

Table 9-1. Sedimentation Rates

% of Biomass Sedimenting at Rates ≥ V cm/hr

	S. quad. Batch (clumped)		S. quad 2 stage	S. quad Contin.	S.So2a Batch		Ankist. Batch
	Unind.	Induc.	Induc.	Unind.	Unind.	Induc.	Unind.
V cm/hr	9-1	9-8	9-16 9-25	9-8 9-25	10-3	10-7	10-7
300	40	40	--	--	--	--	--
150	70	65	--	--	--	--	--
100	70	70	--	--	0	10	5
60	85	80	10-20	0-5	--	25	15
45	--	--	--	--	5	30	25
30	90	90	30-50	10-20	10	35	45
15	--	--	65-75	40-50	--	--	--

Since a sedimentation rate of 30 cm/sec may allow for economic algae removal, the increase in the amount of biomass settling at this rate when nitroven-starved, could be significant.

Slurry density is another sedimentation parameter that was measured in certain instances. Table 9-2 lists culture, sedimentation slurry and supernatent densities measured during some of the sedimentation tests. Concentration facators were 50-100 fold, indicating that compaction of the biomass was good.

Table 9-2. Sedimented Slurry Biomass Densities

		V cm/sec	Cult. Dens. gm/L	Slur. Dens. gm/L	Super-natent Dens. gm/L
S. quadricauda batch					
Uninduced	9-1	60	0.275	25.2	.023
	9-14	60	0.680	38.5	.103
	2-13	60	0.405	25.0	.100
Induced	9-8	60	0.515	29.6	.070
S. quadricauda continuous					
	9-8	15	0.140	24.5	.080
	9-25	15	0.178	15.0	.073
	9-30	30	0.174	13.0	.105
S. quadricauda continuous effluent					
	9-15	15	0.325	22.5	.055
	9-25	15	0.398	14.0	.140
Ankistrodesmus	9-30	30	0.145	11.0	.05
	10-7	30	0.210	25.0	.09

9.3 HARVESTS OF MASS CULTURES

In several instances, cultures from 100 m^2 and 200 m^2 ponds were gravity fed into the 2m deep sedimentation ponds, allowed to settle for 8-24 hrs. and then decanted with a floating pump. Table 9-3 shows results of these tests. The cultures used were all highly clumped. Settling times could have been as short as 4-6 hrs. with no decrease in efficiency. A ten hour settling time is equivalent to a rate of 20-30 cm/hr. The slurry densities listed are based on the measurements taken immediately upon pumping slurries out of the sedimentation ponds and into a 250 gallon tank. Further compaction upon settling would easily double the densities shown.

The Chlorella cultures in general did not settle well at all. However, if a population of rotifers was allowed to develop, the cells clumped. The cultures, when harvested, then exhibited the efficiency of settling shown in the table.

Table 9-3. Harvesting Mass Cultures

Organism	Pond Density ppm	Effl. Density ppm	Slurry Density ppt	Sed. Time hrs.	Harv. Eff. %
S. quad.	868	9	9.0	9	99
	840	21	9.9	8	98
	670	5	8.0	2	99
	724	55	10.3	6	93
	696	87	7.4	12	87
	569	13	10.4	12	98
	329	19	10.2	8	94
	472	36	13.7	24	92
	450	18	13.0	12	96
Chlorella	410	36	11.1	12	91
	368	52	7.6	10	86

SECTION 10.0

OPERATING COSTS

10.1 INTRODUCTION

The data shown in Section 3.0, indicated that an average production of about 15 gm/m^2/day was sustainable throughout most of the year. Although this figure would probably be higher with better strains grown in the U.S. Southwestern environment, we will use 15 gm/m^2/day as a baseline. During the recycling experiment described in Section 5.0, the inputs to the system, i.e., nutrients, water, and power, were monitored for about a month, during which harvested productivity also averaged 15 gm/m^2/day. Thus the cost per unit biomass, of these inputs, can be calculated. Annualized capital costs, costs of labor, and biomass processing are not included in the discussion below.

10.2 CURRENT COSTS OF INPUTS

Table 10-1 summarizes the costs of inputs during the recycle experiment that is being taken as representative of system performance. During this experiment, the depth of the pond was 20 cm, the mixing speed was 30 cm/sec, and the loss of CO_2 at injection was about 34%. An overall input cost of $1.09/kg, AFDW resulted. Most of this was due to the high cost of CO_2 and its inefficient use. Nitrogen contributed 12% of the total cost, at a biomass N content of 12%. The other nutrient costs, detailed in Table 10-2, are higher than would be expected in a large-scale system since 1) many of these chemicals would have to be available in the water resource and 2) bulk purchasing would decrease unit costs of phosphate and iron. Mixing power cost is based on a high channel velocity that, as discussed in Section 6.0, is not necessary when less than 25 gm/m^2/day are being produced.

10.3 PROJECTED COSTS OF INPUTS

Table 10-3, shows the costs of the same factors, but modified to take into account cost reduction possibilities. CO_2 is still the largest cost factor, and is, in fact, still too expensive even at the price of $.05/kg, the best commercial price available now. An increased utilization efficiency is used: only 5% loss at injection and outgassing losses equivalent to 5 gm/m^2/day algal production. Both should be easily achieved when a sump is used for carbonation, when mixing speed is $\leq$ 20 cm/sec, and when the pH range is chosen to avoid excessively high dissolved CO_2 levels. Even so, CO_2 comprises 50% of the total input costs, at $.125/kg algae produced. Since this is about all that the biomass fuel precursor is worth, CO_2 cost must be cut further, at least 50% more. Finding large amounts of low cost CO_2 is probably the greatest single obstacle to lowest cost algal biomass production.

Table 10-1. Initial Algal Biomass Production Costs

28 days, S. quadricauda, effluent recycle, freshwater, $200m^2$

Component	Unit Price,$	Amt. Used	Cost,$	% total cost
CO_2	.275/Kg	3Kg/Kg alg.	68.31	75.9
Urea	.51/Kg	.26Kg/Kg alg.	10.78	12.0
Other Nutr.	----	----	6.71	7.4
Pump power	.06/Kw.hr	5.2Kw.hr	.31	0.3
Mixing power	.06/Kw.hr	$.42W/m^2$	3.38	3.8
Water	11.5/A ft	$91m^2$/day	0.48	0.5
		TOTAL	89.97	99.9

Total biomass harvested: 82.8Kg (15.0 gm/m^2/day av)

Cost: $1.09/Kg AFDW = $.49/lb

Table 10-2 $200m^2$ Effluent Recycle--Media Component Input

Date	Biomass Harv.,Kg	$NaHCO_3$ $0.66/Kg	$MgSO_4$ $0.55/Kg	DKP $2.73/Kg	DSP $1.70Kg	$FeSO_4$ $.88/Kg	Urea $.51/Kg	Water .000 L
5/27	24.6	1.33	.125	.225	.225	.3	6.0	14.4
		.88	.07	.61	.38	.26	3.04	
6/2	20.3	--	.125	.15	.15	.4	5.1	10.8
			.07	.41	.26	.35	2.58	
6/10	19.6	--	.5	.3	.15	.4	5.1	13.2
			.28	.82	.26	.35	2.58	
6/17	18.3	--	.5	.3	.15	.4	5.1	12.0
			.28	.82	.26	.35	2.58	
TOTAL	82.8	1.33	1.25	.975	.675	1.5	21.3	50.4
		.88	.70	2.66	1.16	1.31	10.78	

Top entry: kg used
Bottom entry: cost,$

Table 10-3. Cost Reduction Possibilities

Component	Cost Reduction	Cost,$	% Total	c/kg alg.	Projected c/kg alg.
CO_2	$.275--0.05/kg CO2 3kg/kg -- 2.5kg/kg	10.35	51	12.5	6.2
Urea	.26 kg/kg --.13 kg/kg	5.39	26.6	6.5	3.3
Other chemicals	All but P, Fe available in water, bulk pricing	3.30	16.3	4.0	2.0
Pump Power	-	0.31	1.5	0.37	0.4
Mixing Pwr.	0.42 W/m^2--0.14W/m^2 Effic.: 50%	0.45	2.2	0.54	0.54
Water	-	0.48	2.4	0.69	0.6
	TOTAL	20.28	100	24.6	13.0

The cost of nitrogen has been lowered in the projection due mainly to production of nitrogen limited biomass. If the biomass contained only 6% N, then the ura costs $.065/kg of algae. If the content is lowered further, and some nitrogen is recycled from the biomass processing, then this cost can be halved again. The other chemical costs are limited to iron and phosphate. These must be replenished as algae is produced on recycled water.

Power costs can be lowered by lowering the channel velocity from 30 cm/sec to 15cm/sec. The system must be designed to maintain an overall efficiency of 40-50% for the mixing systems.

10.4 CONCLUSIONS

The total cost of the inputs considered is 13-25 cents per kg AFDW, with the cost of CO_2 making most of the difference within this range. Increased productivity would have little impact on this cost since the productivity related inputs - nutrients - comprise over 90% of the total. With increased productivity CO_2 cost would decrease due to an improved ratio of carbon going into algae vs outgassing (if the mixing speed and CO_2 level are not increased to achieve the higher productivity). But this would still only lead to a slight decrease in CO_2 use, e.g., from 2.5 to 2.2 kg/kg algae produced if productivity were doubled. The major impact of increased productivity comes in annualizing capital costs per unit biomass produced and in distributing labor costs. Since these costs would need to be less than that of the

lowest total cost/kg in Table 10-3 (otherwise the biomass is too expensive for fuel), the impact of increasing productivity is still limited by nutrient costs. Any cost savings from increasing productivity can be gained only when the increases are not accompanied by major increases in capital costs or operating costs.

SECTION 11.0

SUMMARY AND CONCLUSIONS

11.1 PRODUCTIVITY RELATED RESULTS

With a two species rotation - a high temperature Chlorella sp in the summer and a Scenedesmus sp in fall, winter, and spring - a yearly average of 15 gm/m^2/day was obtained in sequential batch, nitrogen sufficient cultivation.

At a location where maximum ambient temperatures are above 25°C for 9-10 months, and minimum ambient temperatures above 10-15°, 20-25 gm/m^2/day could be achieved.

Higher productivities may result using strains pre-screened for:

- tolerance to high DO
- low respiratory rates at high temperature
- tolerance to high irradiance
- broad temperature optimum

Induction of storage products, under nitrogen-depleted conditions has little negative impact on productivity if accomplished within one to three days.

Productivity is independent of mixing speed, for mixing regimes characterized by random turbulence, at least in the range from 1-30 cm/sec up to productivities of at least 25-30 gm/m^2/day.

11.2 COST RELATED RESULTS

Mixing must be sufficient to keep cells in suspension (15-20 cm/sec at most).

Higher mixing speeds require substantially more power input and increase CO_2 losses due to outgassing.

Media recycle is a feasible method for conserving water.

CO_2 is the largest cost factor. Culture operation must be optimized for high productivity (which increases with CO_2 level) and reduced CO_2 outgassing (which increases with CO_2 level and mixing speed). Sources of CO_2 must be found which are both plentiful and below present market prices.

Depending on how it is achieved, increasing productivity, e.g., by a factor of two can only be expected to lower the overall cost of biomass by, e.g., 10-30%, because nutrient input is proportional to biomass output, as are most processing costs. For the most part only annualized capital costs and labor do not necessarily increase with productivity.

11.3 SPECIES SCREENING, MONOCULTURE MAINTENANCE

The more constant the environment, the easier it is to maintain a monoculture.

Very competitive strains will be required to maintain monocultures. TDS and temperature tolerance exert the greatest selection on species, with tolerance to DO and light also important.

Determination of growth response to pH, CO_2 and DO is important in optimizing system output and costs.

Systems must be managed for biomass harvesting, preferably by autoflocculation, or other methods which harvest small cells. Unless a strain is the most competitive by a wide margin, harvesting methods which are not universal will increase the relative competitiveness of non-harvestable organisms when media recycling is practiced.

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APPENDIX

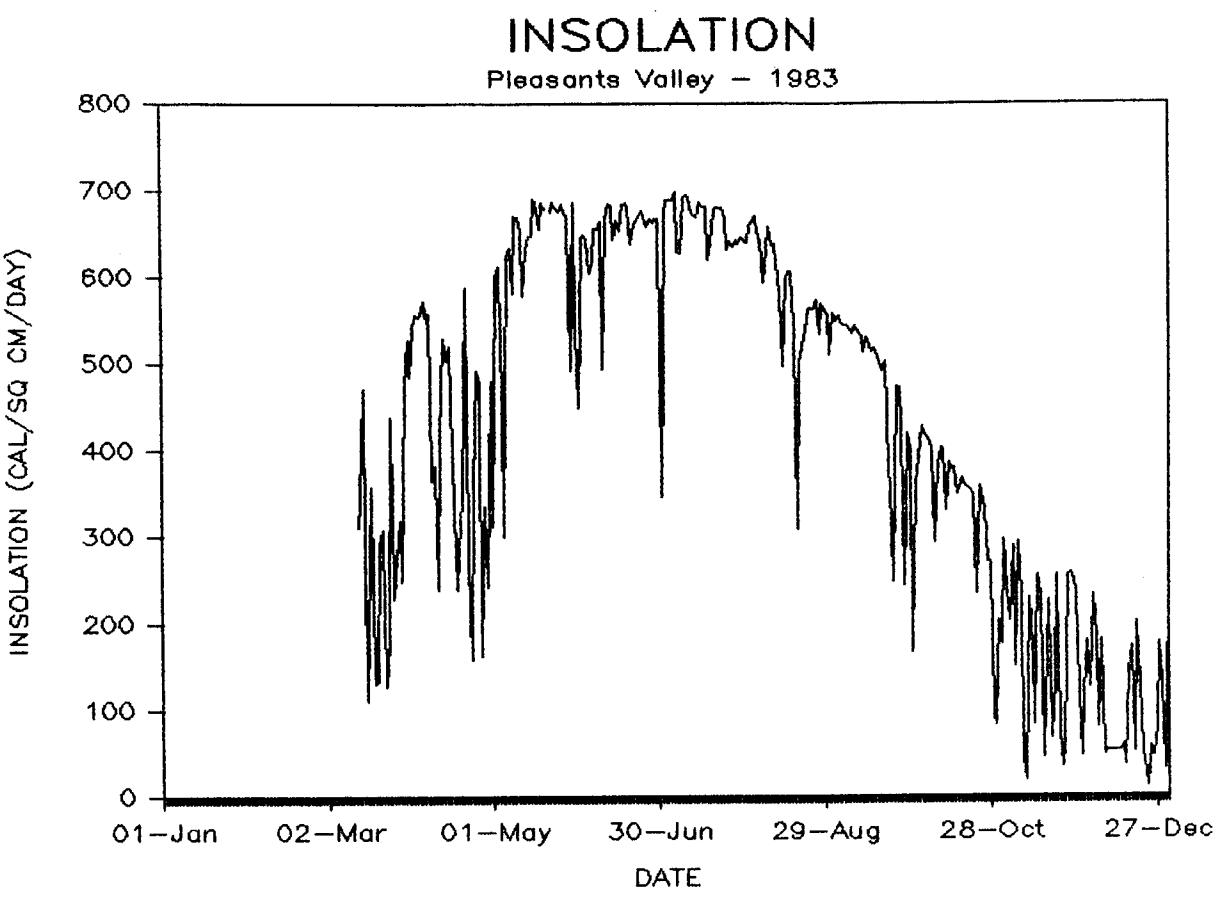
Table A-1. Climate Data: Monthly Averages - 1983

MONTH	INSOLATION		TEMPERATURE (degrees C)			
	Lgy/Day	Std.Dev.	MAX	Std.Dev.	MIN	Std.Dev.
Jan-83						
Feb-83						
Mar-83						
Apr-83	417	128	19	2.2	8	2.8
May-83	616	100	28	5.5	10	2.9
Jun-83	648	51	33	2.7	12	2.6
Jul-83	655	61	34	4.1	13	3.1
Aug-83	581	70	35	3.7	14	3.6
Sep-83	473	100	33	4.7	13	3.3
Oct-83	338	84	29	1.4	9	2.9
Nov-83	170	90	19	4.5	5	3.5
Dec-83	100	65	15	2.6	2	1.3

Table A-2. Monthly Averages - 1984

MONTH	INSOLATION		TEMPERATURE (degrees C)			
	Lgy/Day	Std.Dev.	MAX	Std.Dev.	MIN	Std.Dev.
Jan-84	184	84	21	4.0	2	5.0
Feb-84	286	63	19	1.7	2	2.0
Mar-84	406	92	23	2.4	6	3.2
Apr-84	527	104	24	3.7	6	2.8
May-84	640	79	32	5.1	12	3.7
Jun-84	665	109	33	5.1	12	2.4
Jul-84	656	50	39	4.2	16	3.4
Aug-84	591	78	37	3.6	13	2.8
Sep-84	397	121	35	4.1	13	4.3
Oct-84	250	108	25	4.9	8	3.4

Figure A-1.


INSOLATION
Pleasants Valley – 1983
INSOLATION (CAL/SQ CM/DAY)
800
700
600
500
400
300
200
100
0
01–Jan
02–Mar
01–May
30–Jun
29–Aug
28–Oct
27–Dec
DATE

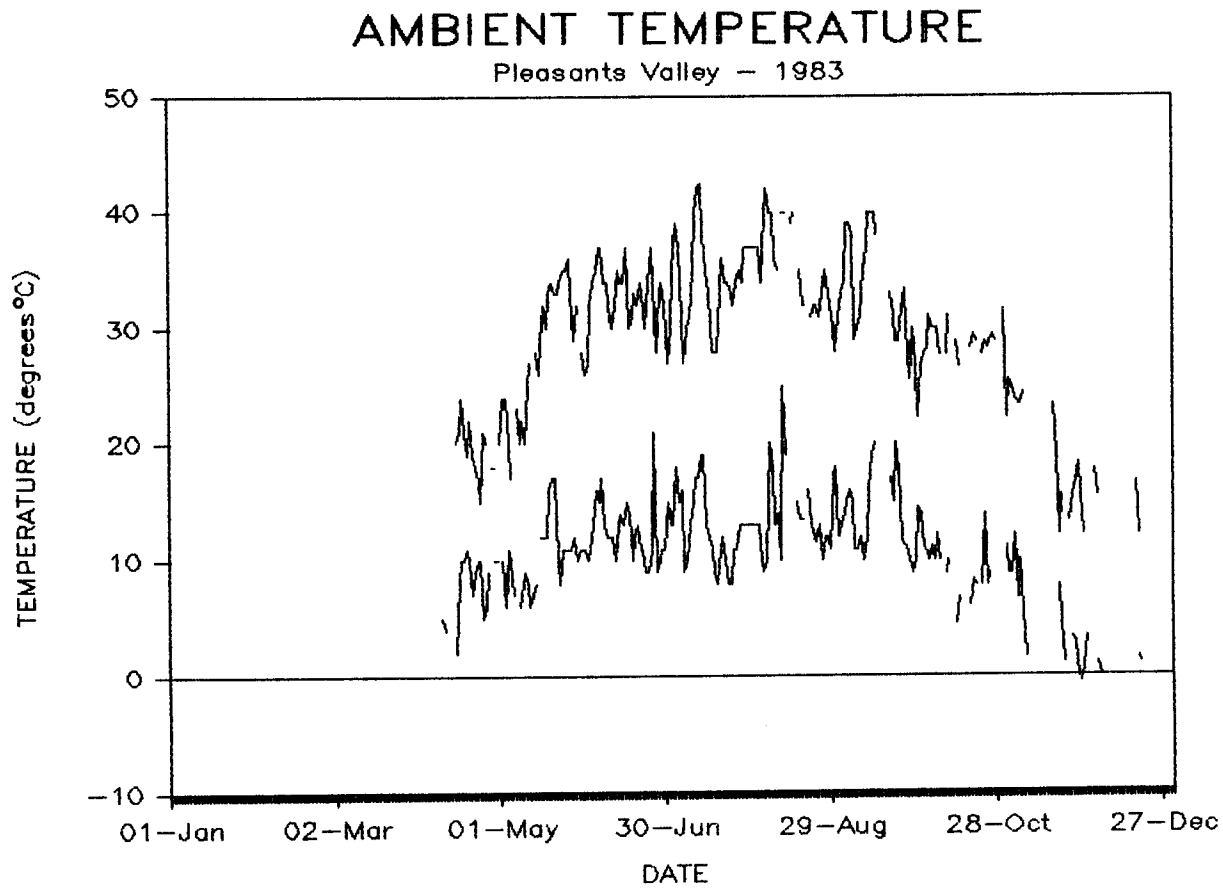
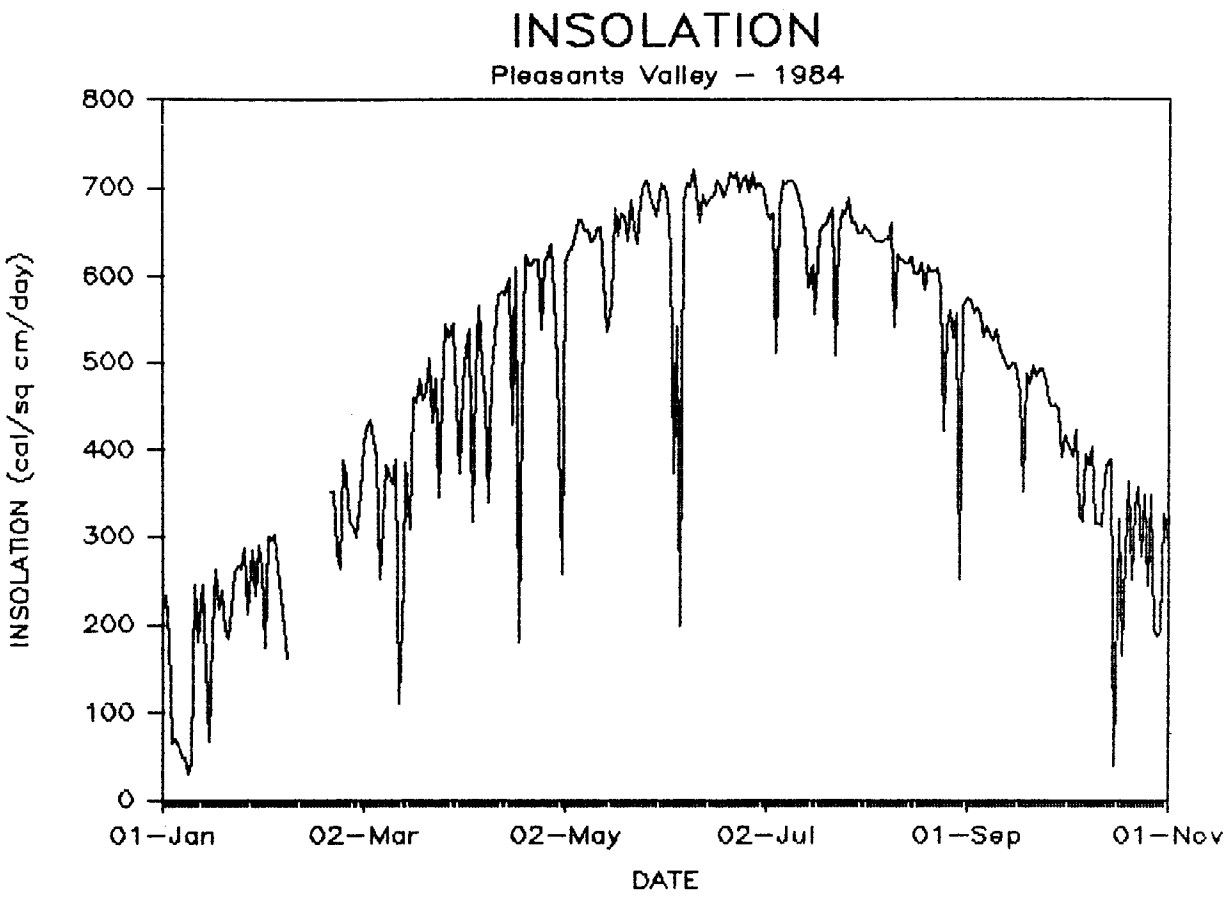

AMBIENT TEMPERATURE
Pleasants Valley – 1983
TEMPERATURE (degrees °C)
50
40
30
20
10
0
–10
01–Jan
02–Mar
01–May
30–Jun
29–Aug
28–Oct
27–Dec
DATE

Figure A-2.


INSOLATION
Pleasants Valley – 1984
INSOLATION (cal/sq cm/day)
800
700
600
500
400
300
200
100
0
01–Jan
02–Mar
02–May
02–Jul
01–Sep
01–Nov
DATE

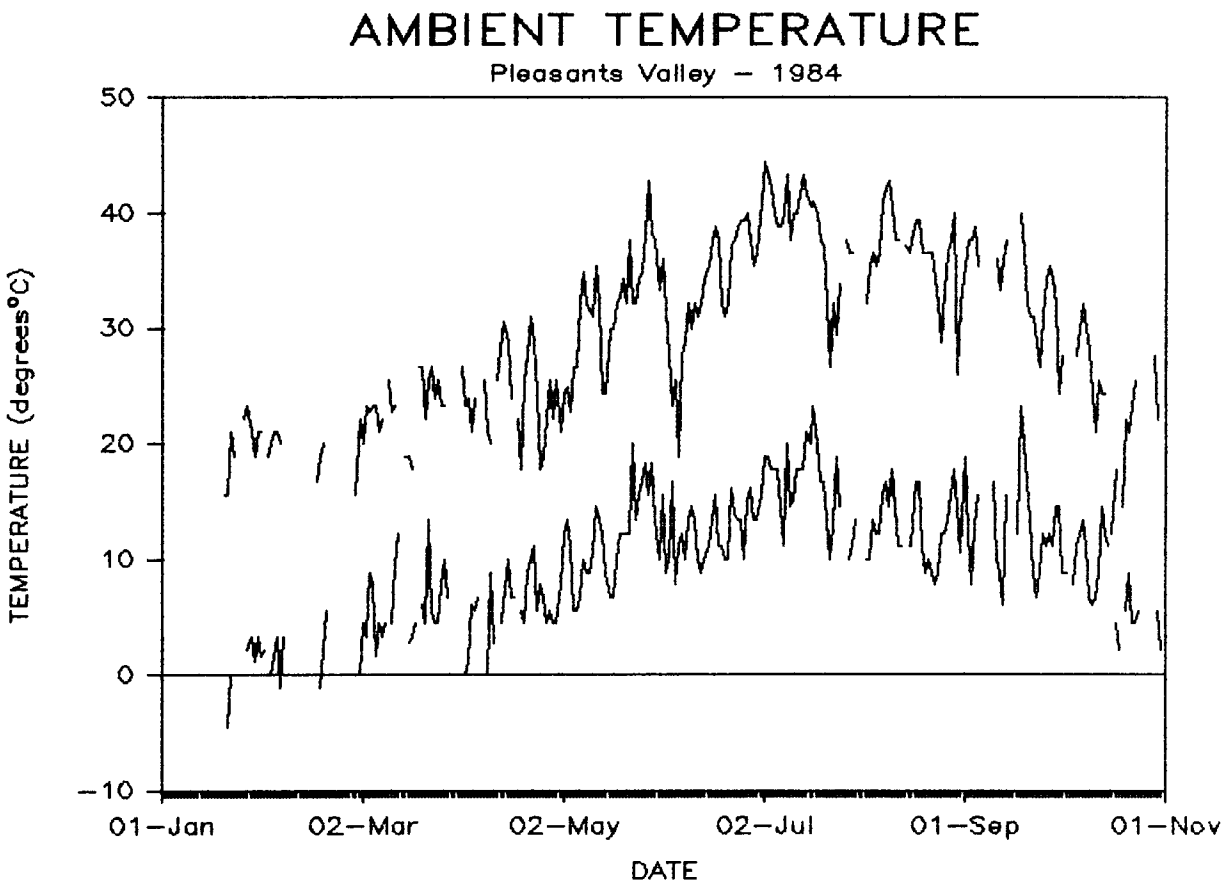
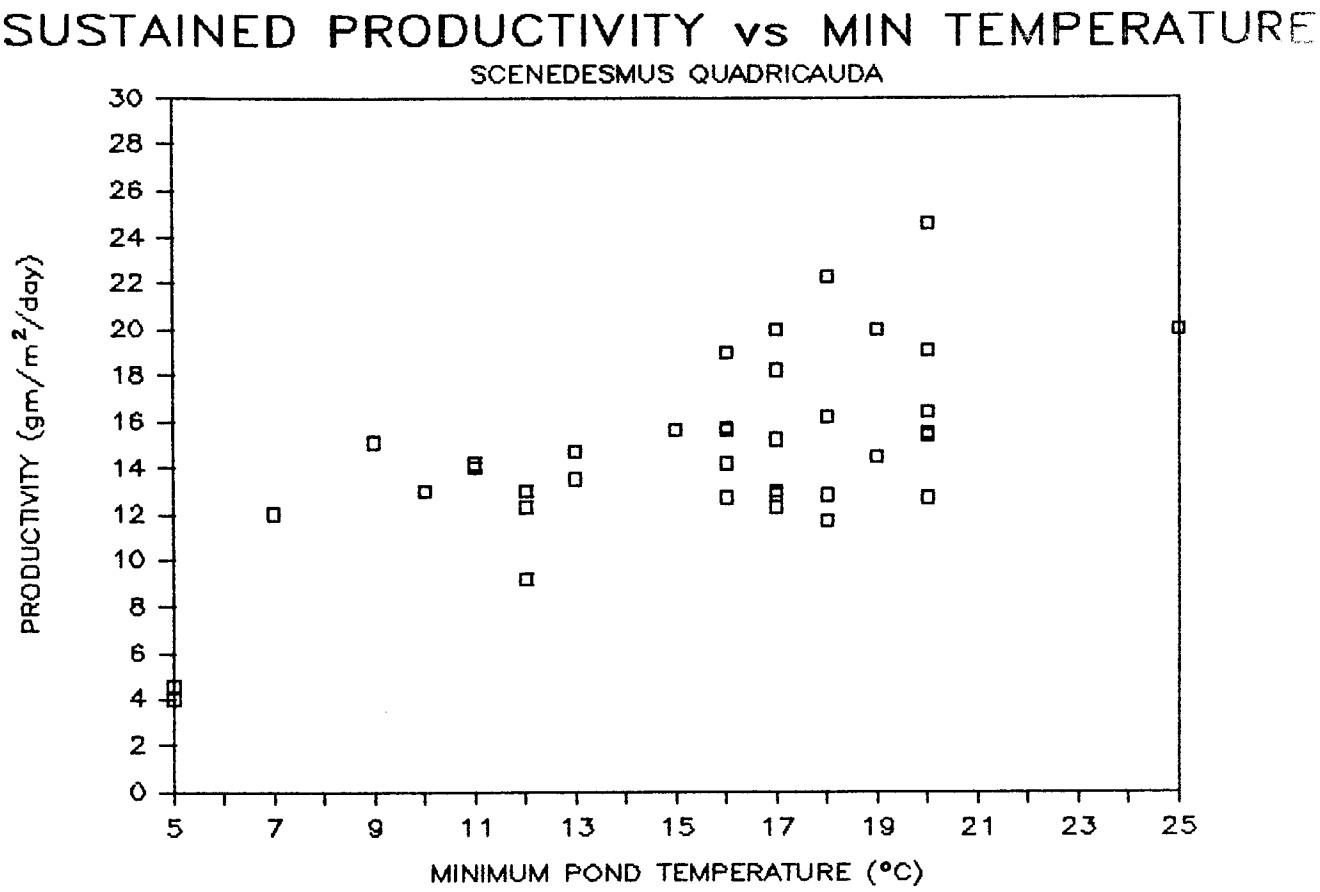

AMBIENT TEMPERATURE
Pleasants Valley – 1984
TEMPERATURE (degrees°C)
50
40
30
20
10
0
–10
01–Jan
02–Mar
02–May
02–Jul
01–Sep
01–Nov
DATE

Figure A-3.


SUSTAINED PRODUCTIVITY vs MIN TEMPERATURE
SCENEDESMUS QUADRICAUDA
PRODUCTIVITY (gm/m²/day)
MINIMUM POND TEMPERATURE (°C)

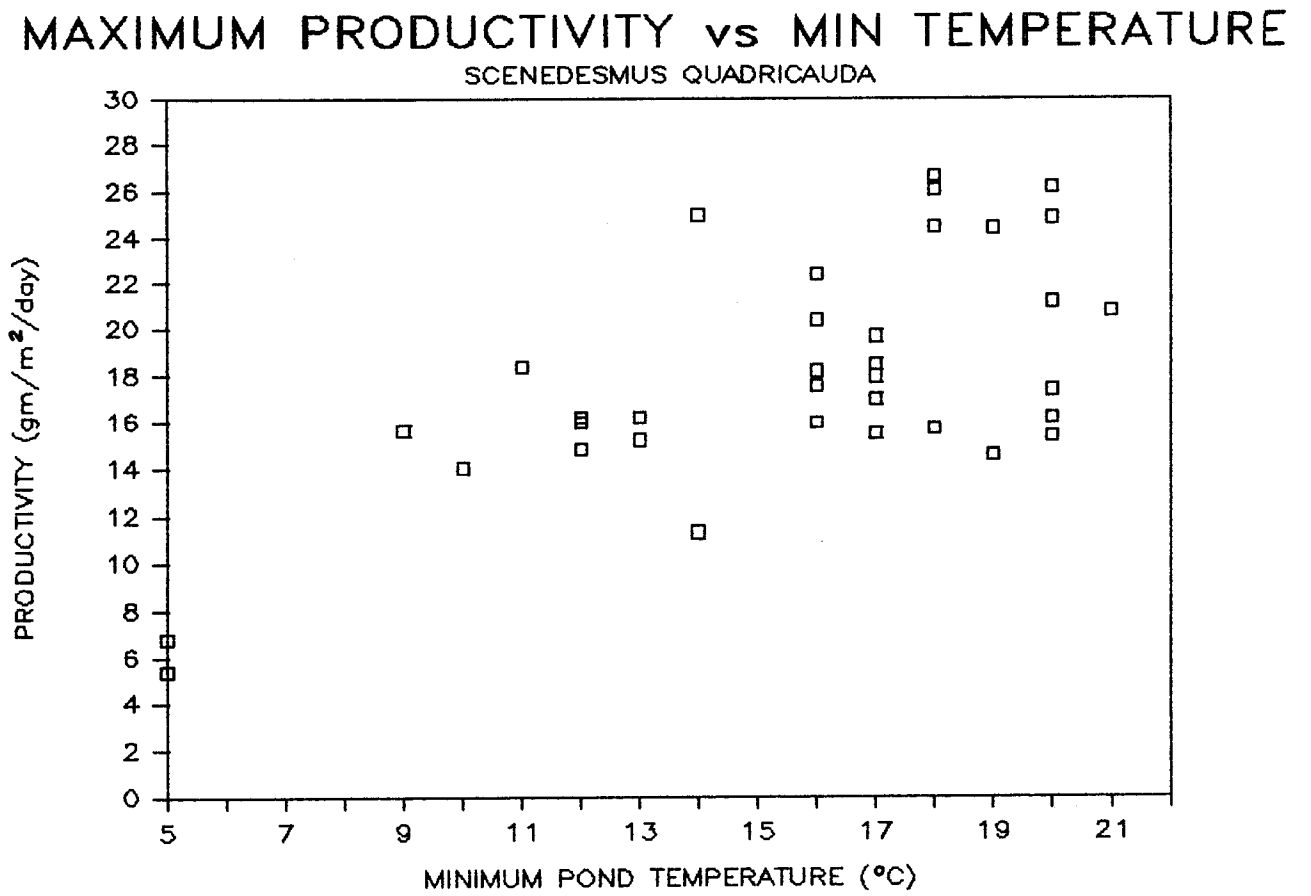
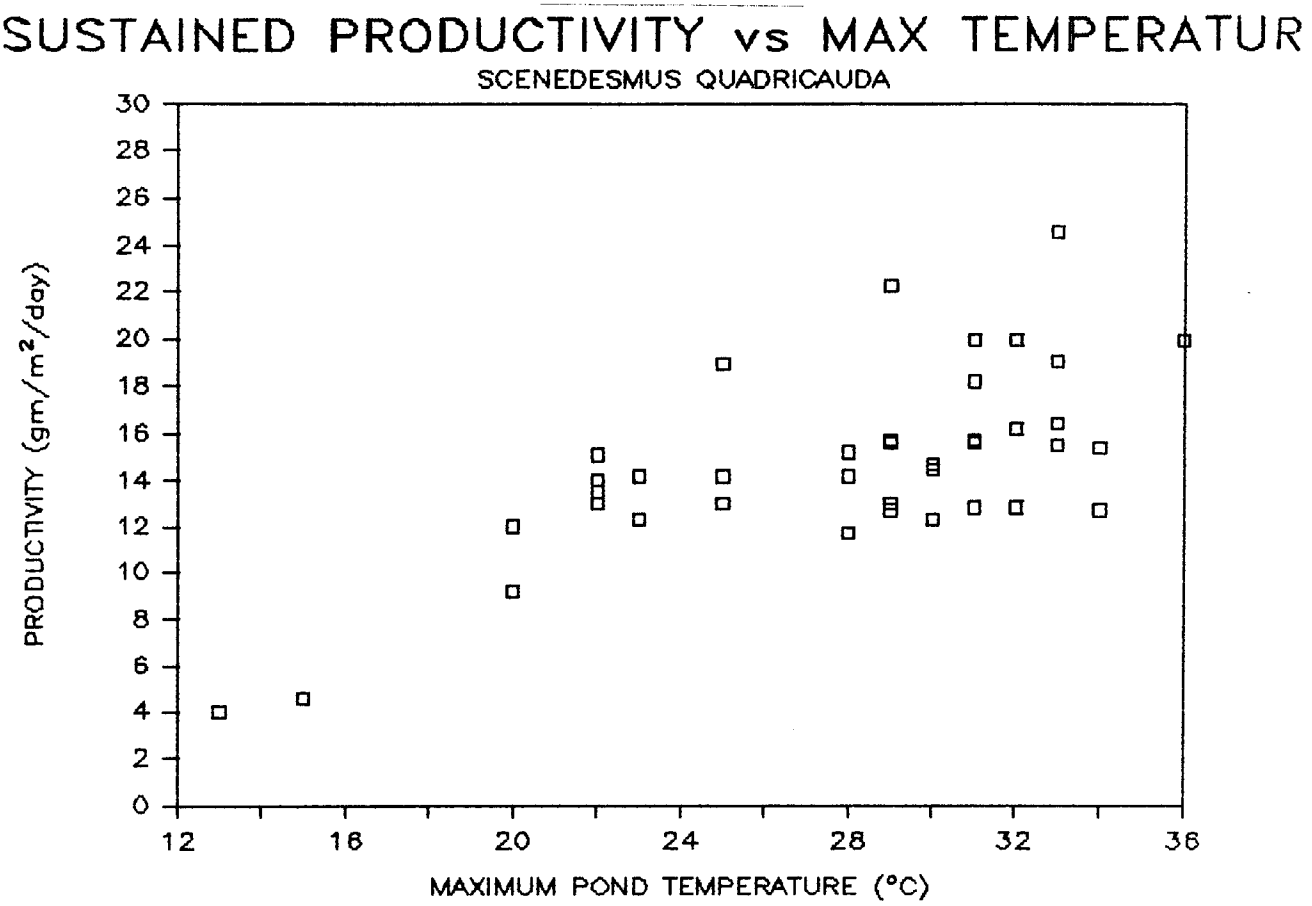

MAXIMUM PRODUCTIVITY vs MIN TEMPERATURE
SCENEDESMUS QUADRICAUDA
PRODUCTIVITY (gm/m²/day)
MINIMUM POND TEMPERATURE (°C)

Figure A-4.


SUSTAINED PRODUCTIVITY vs MAX TEMPERATURE
SCENEDESMUS QUADRICAUDA
PRODUCTIVITY (gm/m²/day)
MAXIMUM POND TEMPERATURE (°C)
0
2
4
6
8
10
12
14
16
18
20
22
24
26
28
30
12
16
20
24
28
32
36

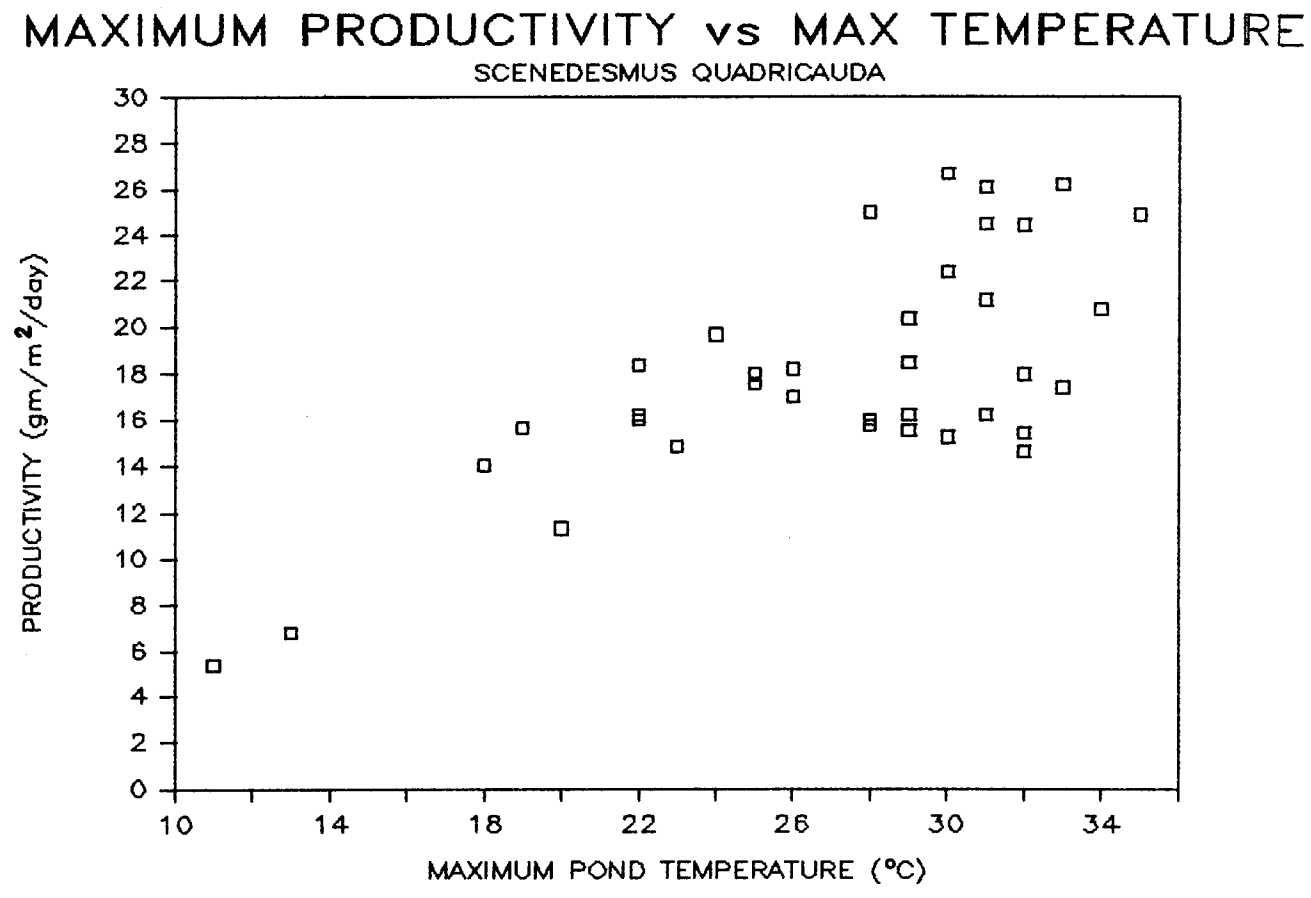
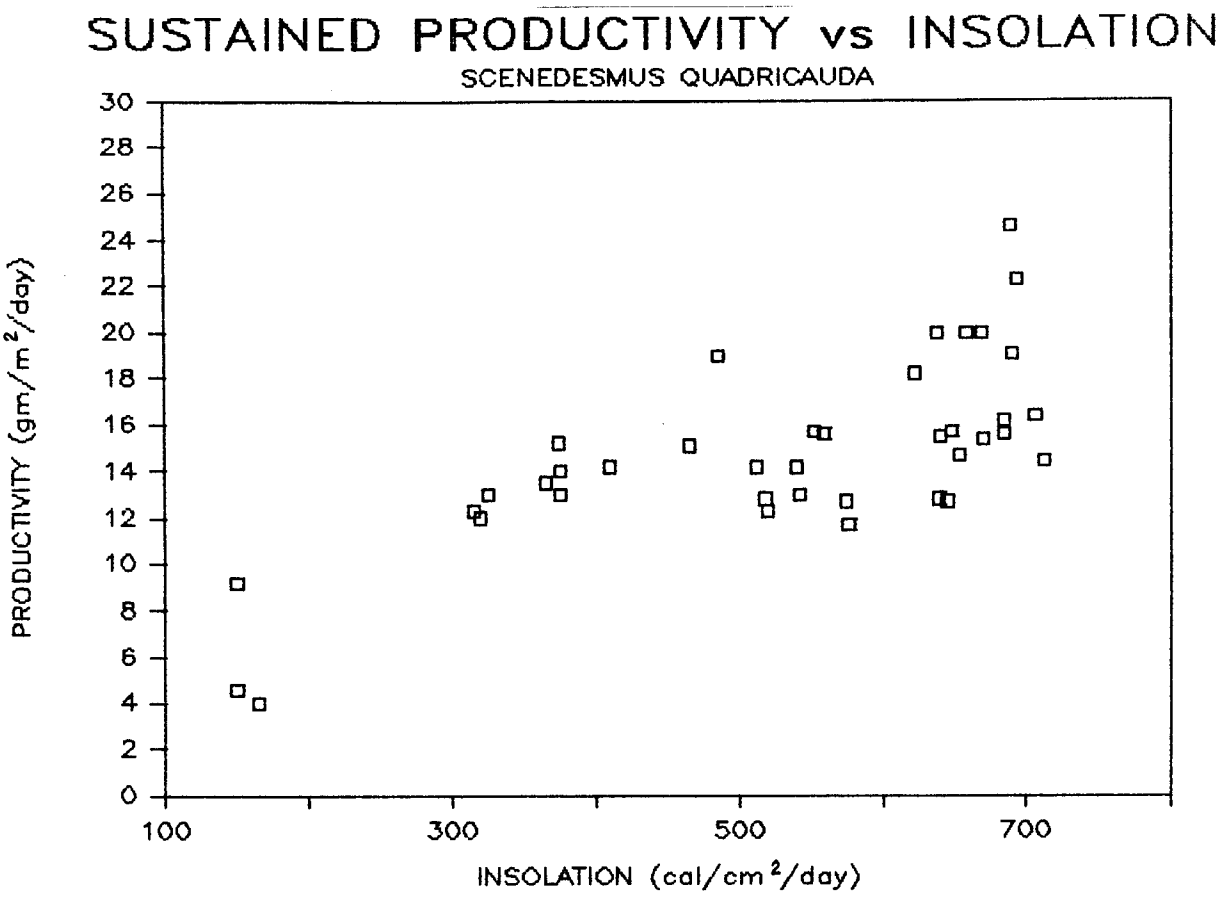

MAXIMUM PRODUCTIVITY vs MAX TEMPERATURE
SCENEDESMUS QUADRICAUDA
PRODUCTIVITY (gm/m²/day)
MAXIMUM POND TEMPERATURE (°C)
0
2
4
6
8
10
12
14
16
18
20
22
24
26
28
30
10
14
18
22
26
30
34

Figure A-5.


SUSTAINED PRODUCTIVITY vs INSOLATION
SCENEDESMUS QUADRICAUDA
PRODUCTIVITY (gm/m²/day)
INSOLATION (cal/cm²/day)
0
2
4
6
8
10
12
14
16
18
20
22
24
26
28
30
100
300
500
700

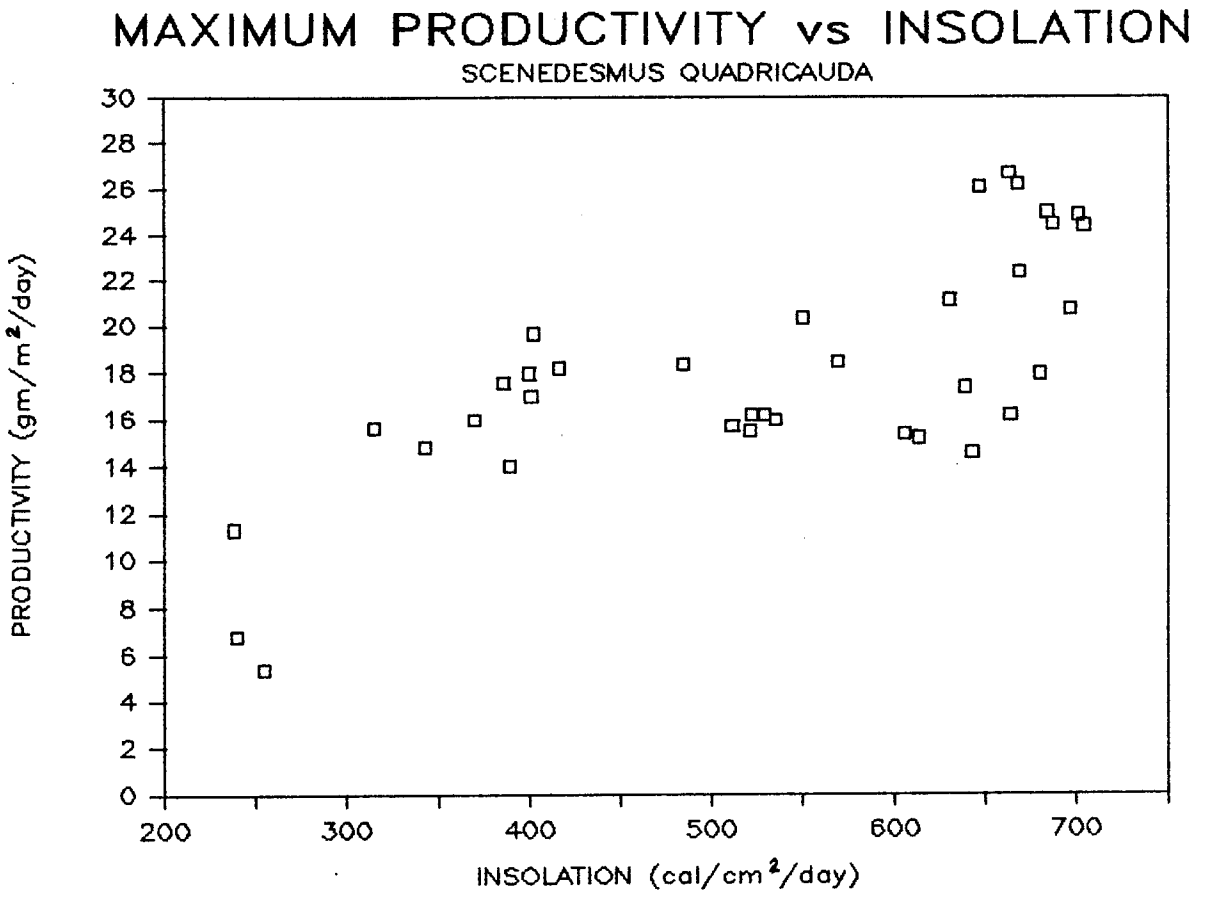
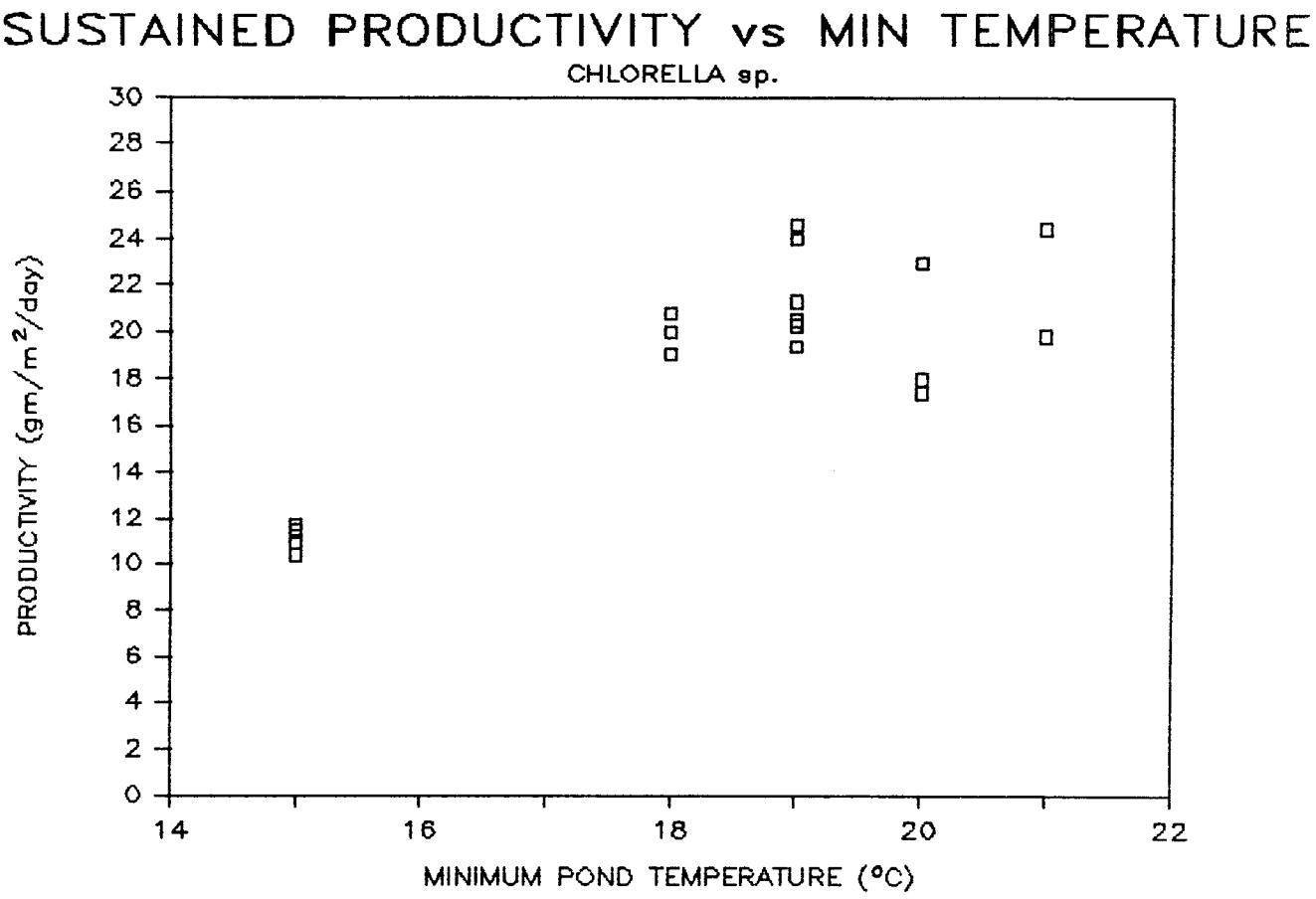

MAXIMUM PRODUCTIVITY vs INSOLATION
SCENEDESMUS QUADRICAUDA
PRODUCTIVITY (gm/m²/day)
INSOLATION (cal/cm²/day)
0
2
4
6
8
10
12
14
16
18
20
22
24
26
28
30
200
300
400
500
600
700

Figure A-6.


SUSTAINED PRODUCTIVITY vs MIN TEMPERATURE
CHLORELLA sp.
PRODUCTIVITY (gm/m²/day)
MINIMUM POND TEMPERATURE (°C)
0
2
4
6
8
10
12
14
16
18
20
22
24
26
28
30
14
16
18
20
22

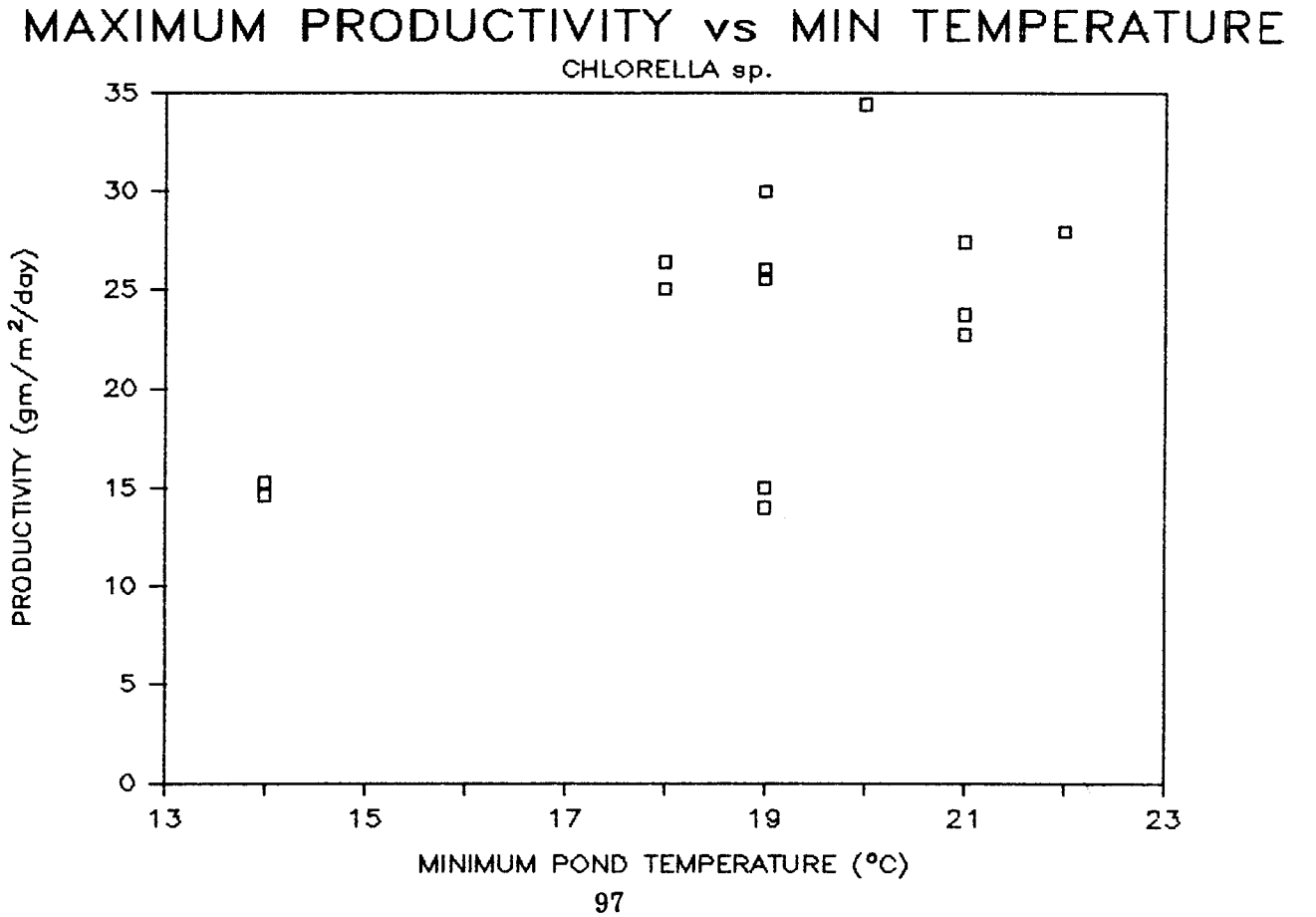
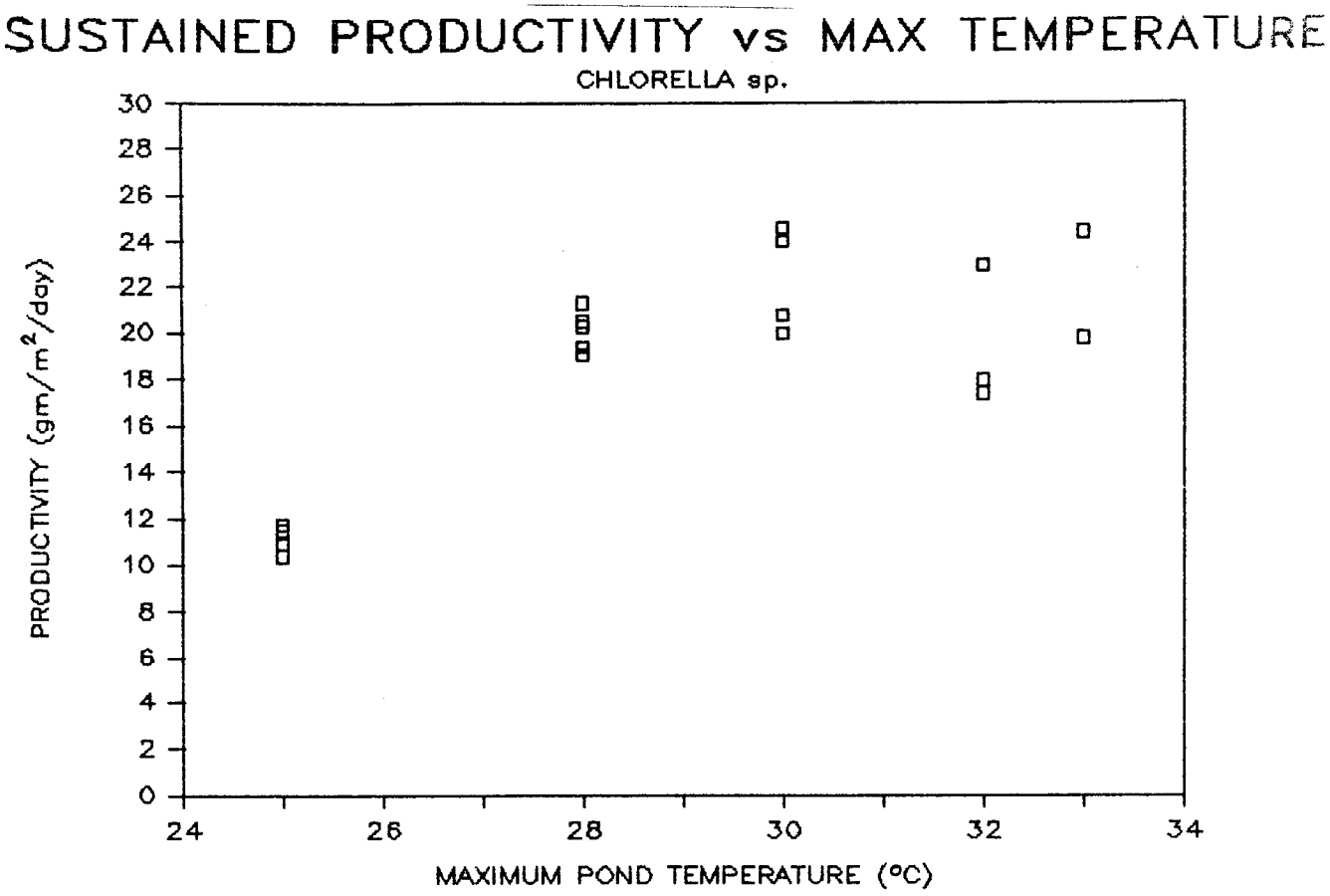

MAXIMUM PRODUCTIVITY vs MIN TEMPERATURE
CHLORELLA sp.
PRODUCTIVITY (gm/m²/day)
MINIMUM POND TEMPERATURE (°C)
0
5
10
15
20
25
30
35
13
15
17
19
21
23

Figure A-7.


SUSTAINED PRODUCTIVITY vs MAX TEMPERATURE
CHLORELLA sp.
PRODUCTIVITY (gm/m²/day)
0
2
4
6
8
10
12
14
16
18
20
22
24
26
28
30
24
26
28
30
32
34
MAXIMUM POND TEMPERATURE (°C)

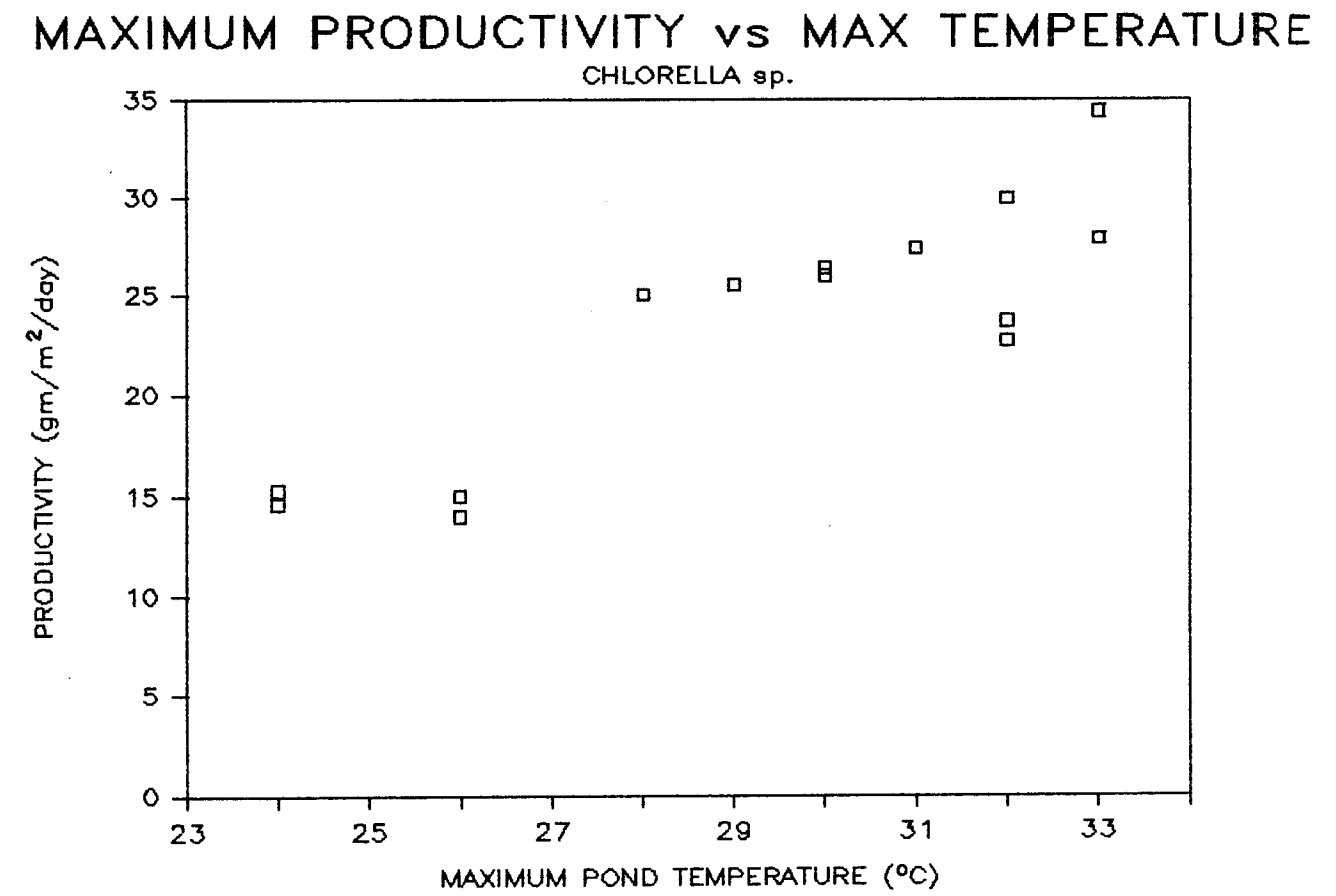
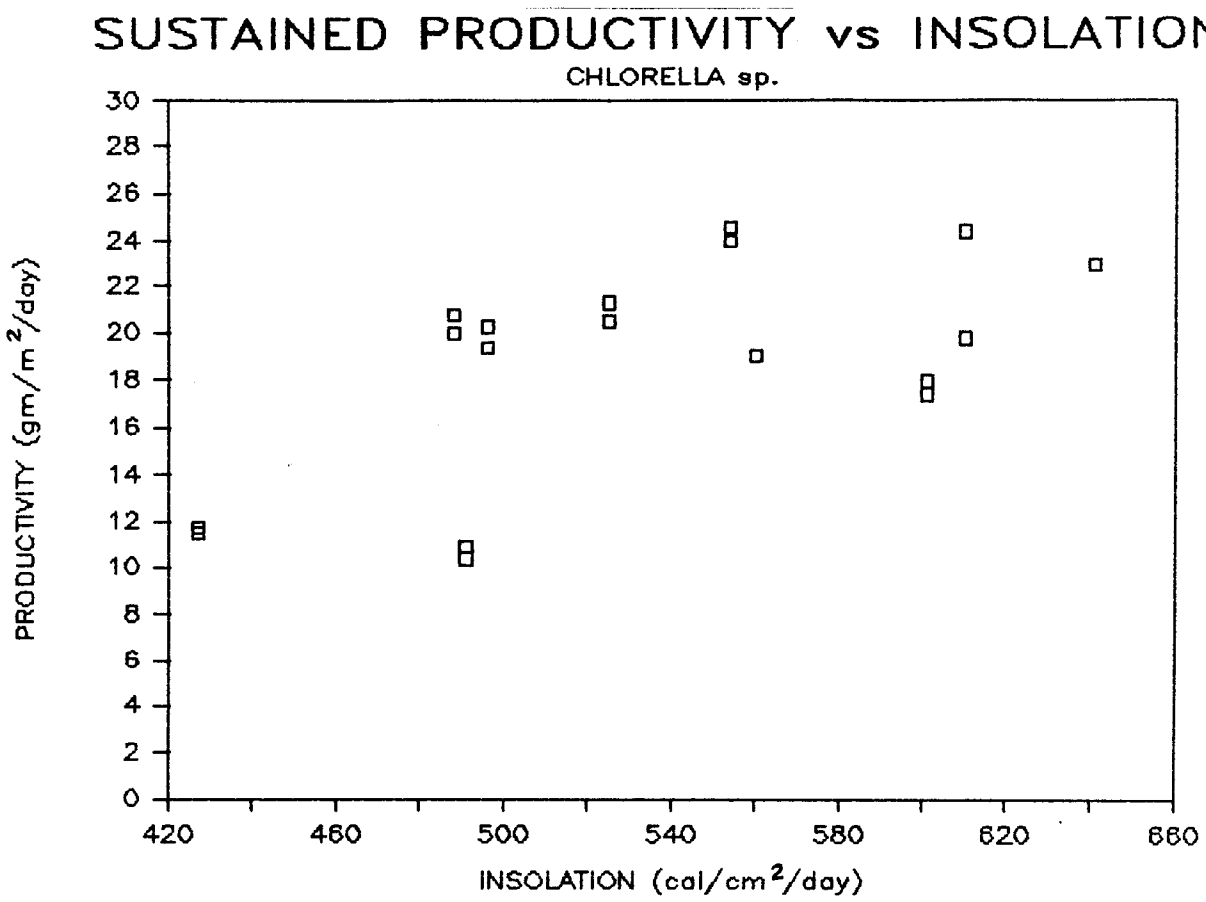
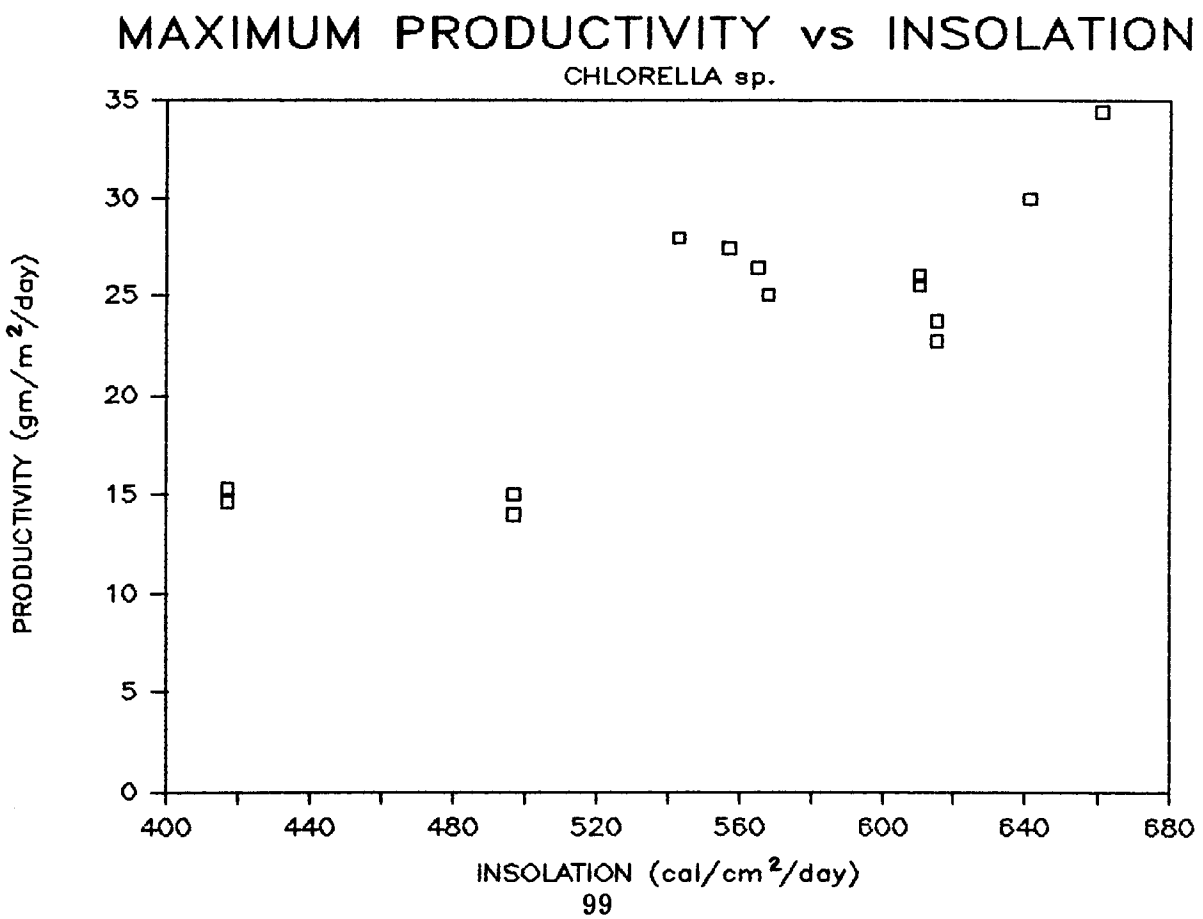

MAXIMUM PRODUCTIVITY vs MAX TEMPERATURE
CHLORELLA sp.
PRODUCTIVITY (gm/m²/day)
0
5
10
15
20
25
30
35
23
25
27
29
31
33
MAXIMUM POND TEMPERATURE (°C)

Figure A-8.


SUSTAINED PRODUCTIVITY vs INSOLATION
CHLORELLA sp.
PRODUCTIVITY (gm/m²/day)
INSOLATION (cal/cm²/day)
0
2
4
6
8
10
12
14
16
18
20
22
24
26
28
30
420
460
500
540
580
620
660


MAXIMUM PRODUCTIVITY vs INSOLATION
CHLORELLA sp.
PRODUCTIVITY (gm/m²/day)
INSOLATION (cal/cm²/day)
0
5
10
15
20
25
30
35
400
440
480
520
560
600
640
680

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16. Abstract (Limit: 200 words)
Of the strains of microalgae provided by other Aquatic Species Program subcontractors, none could out-compete invading wild type algae. Nonetheless, two strains--Ankistrodesmus falcatus and Scenedesmus SO2a--were grown outdoors in mass culture, one for two months and the other for one month. Two wild type organisms, Scenedesmus quadricauda and Chlorella sp., provided most of the outdoor results. The Scenedesmus was maintained for thirteen months, averaging about 13 g/m^2 day. It was used to compare batch and continuous cultivation for mixing speed experiments, media recycling, storage product induction tests, and the determination of operational costs. Chlorella was grown for two months at over 20 g/m^2 day and was used to determine the relationship between mixing velocity and productivity. Experimental results indicated that organisms responded very differently to pH and CO_2 concentration, as well as to dissolved oxygen. The latter may turn out to be an important screening criterion since high dissolved oxygen is endemic to large systems. The pH should be as low as possible to obtain the highest dissolved CO_2 required for maximizing average production. But this must be balanced against outgassing loss of CO_2, which increases with concentration. Mixing speed affects the surface mass transfer coefficient.

17. Document Analysis
a. Descriptors Algae ; Carbon Dioxide ; Chlorella ; Cost ; Cultivation ; Ponds ; Productivity ; Scenedesmus ; Sedimentation ; Unicellular Algae

b. Identifiers/Open-Ended Terms

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