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SUSTAINABLE BIOREACTOR SYSTEMS FOR PRODUCING HYDROGEN

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Abstract

The overall goal of Hawaii's BioHydrogen Program is to generate hydrogen from water using solar energy and microalgae under sustainable conditions. Specific bioprocess engineering objectives include the design, construction, testing and validation of a sustainable photobioreactor system. Specific objectives relating to biology include investigating and optimizing key physiological parameters of cyanobacteria of the genus *Arthrospira (Spirulina)*, the organism selected for initial process development. Another objective is to disseminate the Mitsui-Miami cyanobacteria cultures, now part of the Hawaii Culture Collection (HCC), to other research groups.

Our approach is to use a single organism for producing hydrogen gas from water. Key stages are the growth of the biomass, the dark induction of hydrogenase, and the subsequent generation of hydrogen in the light. The biomass production stage involves producing dense cultures of filamentous, non-heterocystous cyanobacteria and optimizing biomass productivity in innovative tubular photobioreactors. The hydrogen generation stages entail inducing the enzymes and metabolic pathways that enable both dark and light-driven hydrogen production. The focus of Year 1 has been on the construction and operation of the outdoor photobioreactor for the production of high-density mass cultures of *Arthrospira*. The strains in the Mitsui-Miami collection have been organized and distributed to other researchers who are beginning to report interesting results. The project is part of the International Energy Agency's biohydrogen program.

Introduction

Over the past 25 years, advances have been made in the elucidation of the complex physiological, biochemical, and genetic processes underlying the hydrogen evolution capabilities of microalgae and bacteria. In the area of engineering research and system integration, however, little progress has been made. In fact, no hydrogen-evolving process that showed promise has been demonstrated in the field for any length of time, let alone commercialized. Even those processes that have shown promise in the laboratory have not been evaluated in field performance. Importantly, the solar conversion efficiencies of these systems were below 3%.

Objective

The overall goal of Hawaii's BioHydrogen Program is to generate hydrogen from water using solar energy and microalgae under sustainable conditions. Specific bioprocess engineering objectives include the design, construction, testing and validation of a sustainable photobioreactor system. Objectives relating to biology include investigating and optimizing key physiological parameters of cyanobacteria of the genus *Arthrospira (Spirulina)*, the primary organism of choice. Another objective is to disseminate the Mitsui-Miami cyanobacteria cultures, now part of the Hawaii Culture Collection (HCC), to other research groups.

The effort is a 3-year project. Several areas of research and tasks need to be pursued. These include: (1) sustainable bioreactor system design; (2) hydrogen-producing microbes for the bioprocess; and (3) maintenance of the HCC. The realization of the envisioned process will require the joint development of both the hardware, namely reliable and inexpensive bioreactors, and system "software," namely the cyanobacterial strains that are able to grow and produce hydrogen effectively.

Approach

Our approach uses a single organism for producing hydrogen gas from water. Key stages are the growth of the biomass, the dark induction of the hydrogenase system, and the subsequent generation of the hydrogen in light. The biomass production stage involves producing dense cultures of filamentous, non-heterocystous cyanobacteria and optimizing biomass in innovative tubular photobioreactors, work being done in collaboration with M. Tredici, University of Florence (Tredici and Materassi, 1992). The hydrogen generation stages entail inducing the enzymes and metabolic pathways that enable both dark and light-driven hydrogen production. Our system is also amenable to a single-stage indirect process whereby hydrogen can be produced in the same bioreactor configuration.

Cyanobacteria, like other algae, induce a hydrogen-evolving system under dark anaerobic conditions. Cyanobacteria can accumulate large quantities of carbohydrates when limited for nitrogen. The ability to grow cyanobacteria in large-scale (> 10 ha) has already been demonstrated. This project will initially utilize the HCC as the primary source of cyanobacteria that should be suitable for the process. Strains will be screened for key characteristics such as carbohydrate accumulation (total, productivity), dark-anaerobic hydrogenase induction (rate, maximal), and light-driven hydrogen evolution (effects of light, extent of utilization of stored carbohydrates, inhibition by oxygen).

Technical Goals

Technical goals include: (1) Designing, constructing, and testing tubular photobioreactors that offer superior operational characteristics yet are inexpensive to construct and operate for hydrogen production. Specific goals are demonstrating high productivities, effective mass transfer and outdoor hydrogen production. (2) Understanding and manipulating the physiological parameters of the microalgae to optimize for hydrogen production. The main near-term technical goal is to test the tubular bioreactors performance with *Arthrospira* strains

Major Barriers to Meeting Technical Goals

Major barriers: (1) Understanding the physiological aspects of hydrogen production by these strains to permit developing protocols for effective hydrogen production in the lab; (2) Applying and adapting such protocols to photobioreactors, i.e., integrating the biology with engineering.

Past Results

This research on photobioreactor systems and cyanobacterial hydrogen production has completed its initial year. A previously supported project involved the transfer of the Mitsui-Miami collection to Hawaii.

Current Year Accomplishments and Status

This project was initiated in February 1997 with the design and construction of the prototype photobioreactors at the Kewalo Basin site in Honolulu. An automated monitoring and control system for culture temperature and pH was designed and implemented. Dissolved oxygen and light intensity are also automatically monitored and recorded. The photobioreactors and key components of the monitoring and control systems are shown in Figures 1 and 2. Further improvements in bioreactor design and operations are in progress. An engineering analysis of reactor performance has been initiated; for example, Figure 3 shows sample data for the relationship between holdup and gassing rate. Analyses of mass transfer coefficients and hydraulic dispersion coefficients are planned.

The operational characteristics of the tubular photobioreactors in Hawaii were tested with *Arthrospira*, an organism known to contain hydrogenase and produce hydrogen in the dark. The initial work developed protocols for high-density, high-productivity cultures under local conditions of light and temperature and compared these with prior experience in Italy. *Arthrospira* cultures have been successfully adapted to full sunlight by means of a stepwise process using shade cloth. Culture densities of at least 7.5 g/L were achievable during batch growth of adapted cultures (Figure 4). Semicontinuous culture growth was maintained within specified density ranges by varying the replacement rate of the culture medium; this was accomplished by means of an appropriate daily dilution with fresh medium. Biomass production (dry weight) averaged 0.43 g/L/d (1.03 g/L/d maximum) within the biomass ranges 2.6 - 3.5 g/L and 5.1 - 6.1 g/L (Figure 5). Similar productivities were achieved whether dilution was performed in the morning or late afternoon (Table 1).

Figure 6 shows a typical day's insolation curve and culture growth, and illustrates two factors which act to decrease biomass production. A growth slowdown is apparent during the 2-h period in which maximal sunlight intensities were observed, reflecting inhibition of photosynthesis by

high light intensities. An even larger decrease in productivity was caused by the large nighttime biomass loss, amounting to 20% of dry weight in the sample data shown (Fig. 6). Dark respiratory losses of this magnitude would substantially decrease carbohydrate reserves available for hydrogen production. An experiment designed to test whether dark biomass losses could be decreased by means of oxygen limitation at night is shown in Figure 7, while results are summarized in Table 2. Results suggest that oxygen deprivation at night reduced biomass loss, but also decreased production during subsequent illuminated periods. Thus, net 24-hour production was unaltered by nighttime bubbling with nitrogen. Work is underway to determine whether the treatment increased the carbohydrate/protein ratio or otherwise affected cellular composition. Additionally, laboratory research on the physiology of hydrogen production by*Arthrospira* is being initiated.

Table 1. Effect of morning versus afternoon dilution on daily dry weight production by semicontinuously grown cultures.

Day	AM Dilution	PM Dilution
1	0.622	0.514
2	0.518	0.395
3	0.275	0.251
4	0.589	0.667
5	0.149	0.165
6	0.782	0.336
7	0.079	0.560
8	0.359	0.368
9	0.160	0.448
Mean	0.392	0.412

Table 2. Effect of nighttime N_2 gassing on culture productivity and biomass losses.

Nighttime Bubbling	Daylight Production, g/l/d	Dark Biomass Loss, g/l/d	Net Production, g/l/d
N_2	0.547	0.155	0.392
Air	0.659	0.256	0.403
n	5	5	5
P (T <= t)	0.06	0.02	0.82

Data produced by the outdoor experiments will be used in economic and systems analysis. A preliminary analysis of the two-stage biophotolysis process has been carried out that indicated the overall cost of hydrogen production would be below $15/MMBTU, if certain research goals can be met such as the achievement of very high photosynthetic efficiencies (Benemann, 1995). A key issue is the cost of the photobioreactors, both capital and operating. This project will develop information to allow a more detailed cost analysis, in particular for Hawaii which because of favorable environmental conditions is a likely location for the development and application of such systems.

This project has just finished its first year. A manuscript has been prepared and accepted for publication (Szyper et al., 1998). An invited paper to *Trends in Biotechnology* is in preparation. The research was reported at BioHydrogen '97 and at several workshops and meetings. The work was presented at the American Society for Microbiology meeting in May 1998 (Radway et al., 1998). Interest in the tubular photobioreactors and the culture collection is evident. During the year, discussions have been held with various firms and additional contacts are being pursued.

Plans for Future Work

Major actions for Year 2 include the following:

Task 1. Physiology of Hydrogen Production by *Arthrospira.* Specific subtasks include: (a) Hydrogenase induction of light and N-limited cultures (effects of carbohydrate on dark fermentations); (b) Hydrogen evolution in the light by anaerobically adapted cultures.

Task 2. Sustainable Bioreactor System Design. Subtasks include: (a) continuing performance evaluation of the tubular photobioreactors with several cyanobacterial strains; and (b) development of protocols for induction of hydrogenases and hydrogen production in the photobioreactors.

Task 3. Maintenance of HCC Subtasks include: (a) maintenance and curation of the Mitsui-Miami strains and (b) distribution of strains to qualified researchers and groups.

This project contributes to the International Energy Agency (IEA) Hydrogen Program Annex 10B on biological hydrogen production. The project is also a part of a general U.S.-Japan understanding that fosters binational cooperative research in biological hydrogen production. DOE is providing funding for the transfer of the Mitsui-Miami collection, the establishment of a scientific base for the HCC collection, and for the dissemination of the Mitsui-Miami strains to other qualified researchers at national laboratories and industry. Funding from the Research Institute of Innovative Technology for the Earth (RITE) is being used to evaluate photosynthetic bacteria in the Mitsui-Miami collection for active hydrogen production.

Acknowledgment

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During the past year, Mitsufumi Matsumoto, a visiting student from Tokyo University of Agriculture and Technology being funded by Japan's Ministry of Education, participated in the project. Andrew Kato, an undergraduate, is currently associated with the project.

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Tredici, M. R. and Materassi, R., From Open Ponds to Vertical Alveolar Panels: The Italian Experience in the Development of Reactors for the Mass Cultivation of Phototrophic Microorganisms, Journal of Applied Phycology, 4, 221-231 (1992).

Figures

Figure 1. Tredici-design tubular photobioreactor at Kewalo Basin, Honolulu, Hawaii. Two eight-tube bioreactors are shown on the research platform. The bioreactors are 20 m long, with a 4 cm diameter tubing. The formation of bubbles is caused by the injection of compressed air.

Figure 2. Photobioreactor components. a) Degassers, b) Sensor assembly (light, temperatures, pH probe), c) temperature control with automated sprinkler, d) air/CO_2 injection manifold.

Figure 3. Holdup (total air bubble volume in all eight reactor tubes) vs. air input rate. Input air pressure was 3 psi.

Figure 4. Batch growth of *Arthrospira* sp. during adaptation to full sunlight.

Figure 5. Daily production by *Arthrospira* sp. maintained within selected density ranges by means of semicontinuous dilution.

Figure 6. 24-hour light and dry weight curve for semicontinuously maintained culture.

Figure 7. Effect of nighttime N_2 gassing on culture growth.

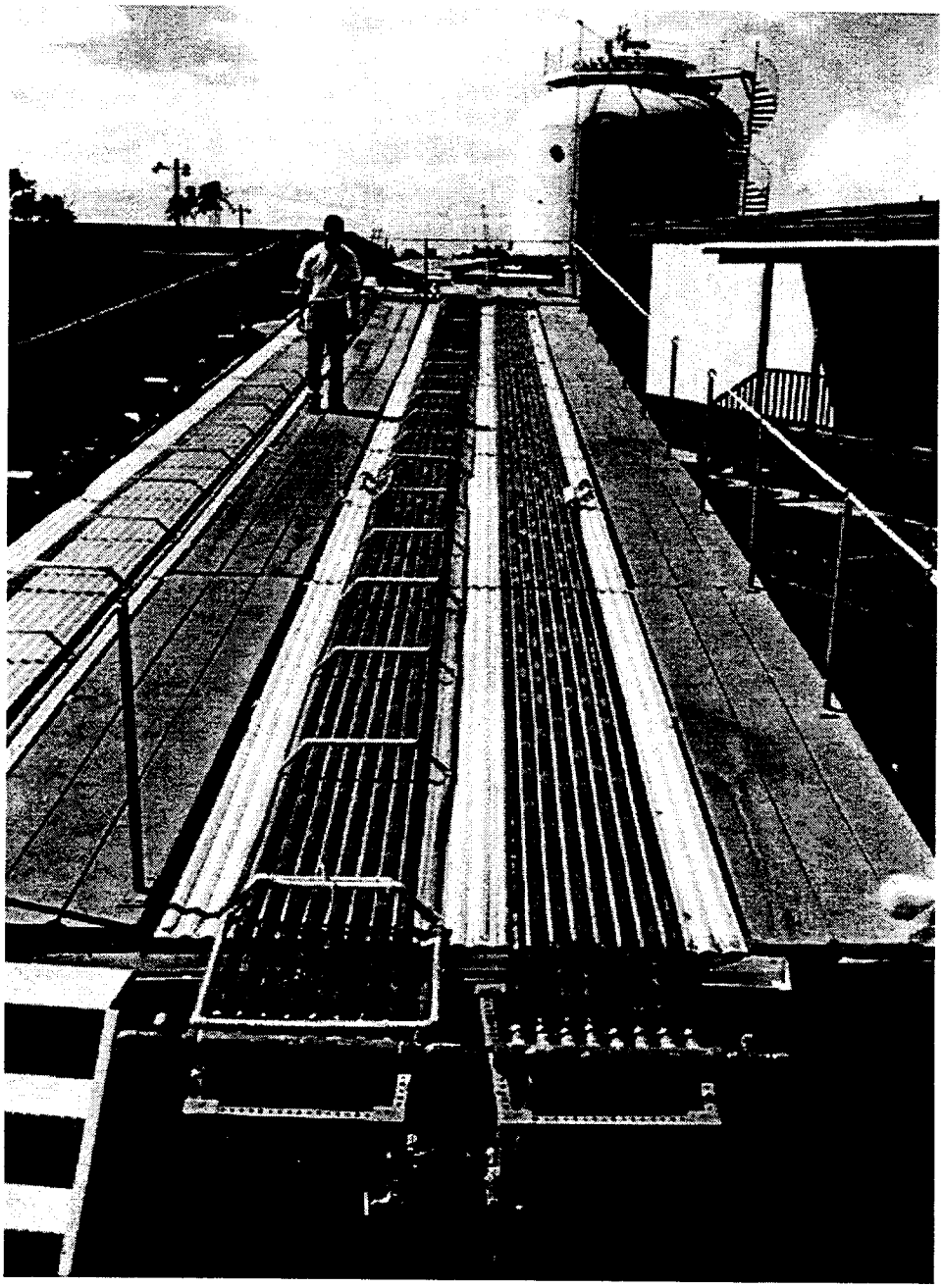

Figure 1

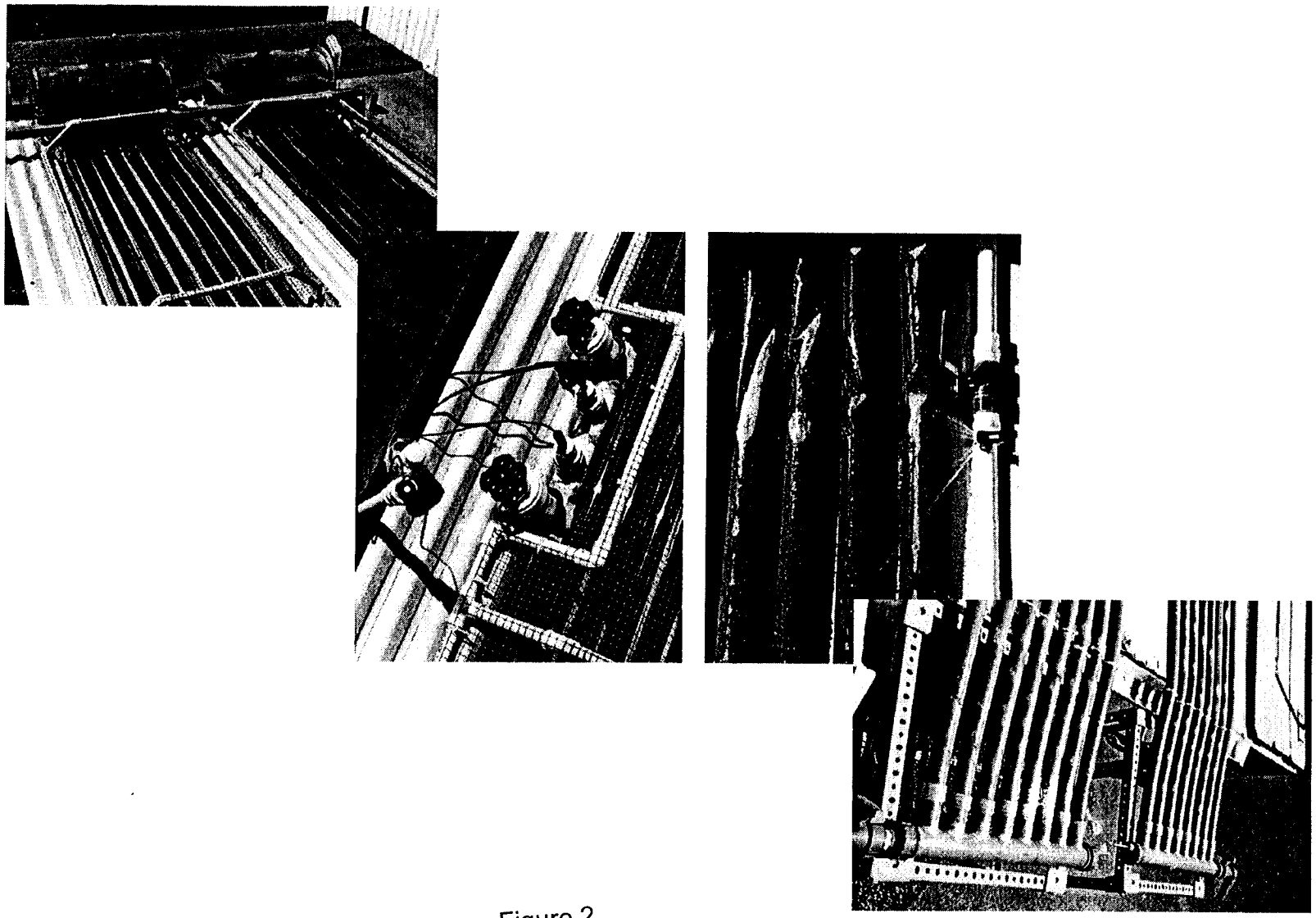

Figure 2

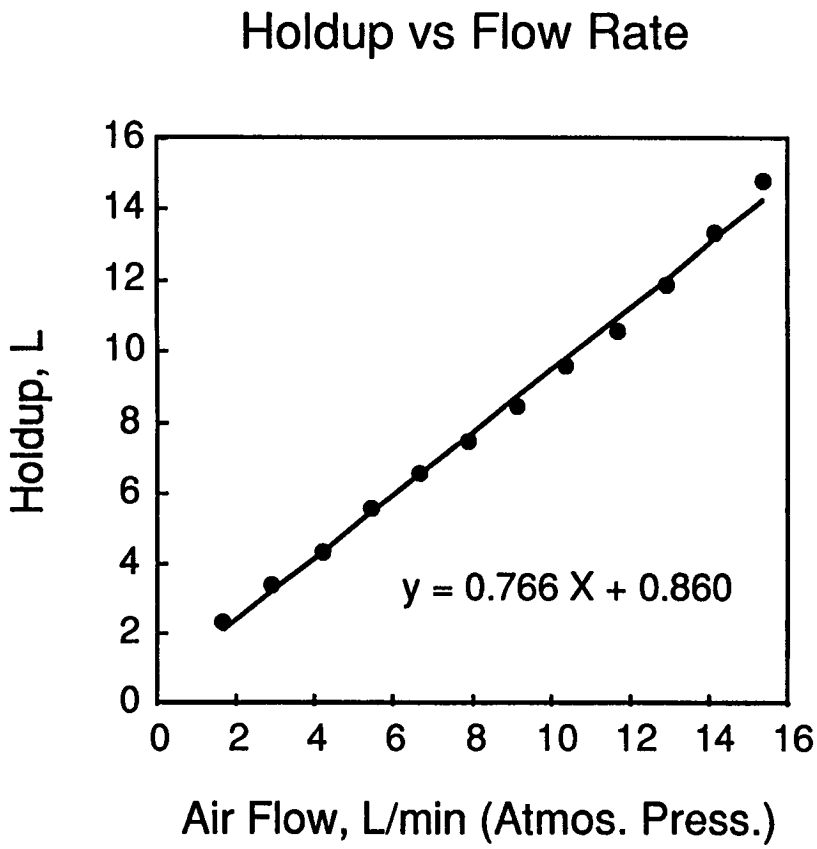
Holdup vs Flow Rate
Holdup, L
16
14
12
10
8
6
4
2
0
0
2
4
6
8
10
12
14
16
y = 0.766 X + 0.860
Air Flow, L/min (Atmos. Press.)

Figure 3

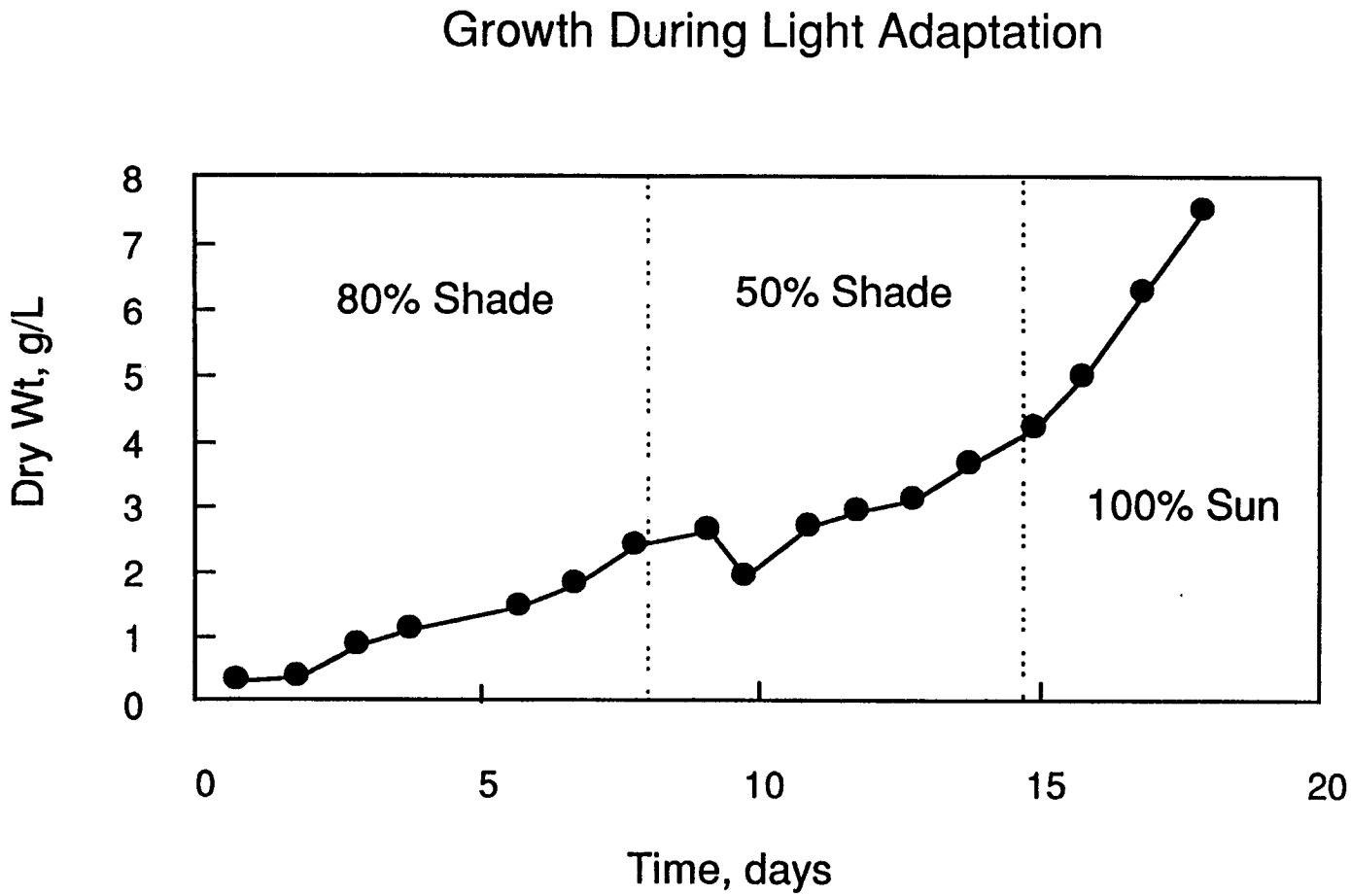
Growth During Light Adaptation
Dry Wt, g/L
8
7
6
5
4
3
2
1
0
80% Shade
50% Shade
100% Sun
0
5
10
15
20
Time, days

Figure 4

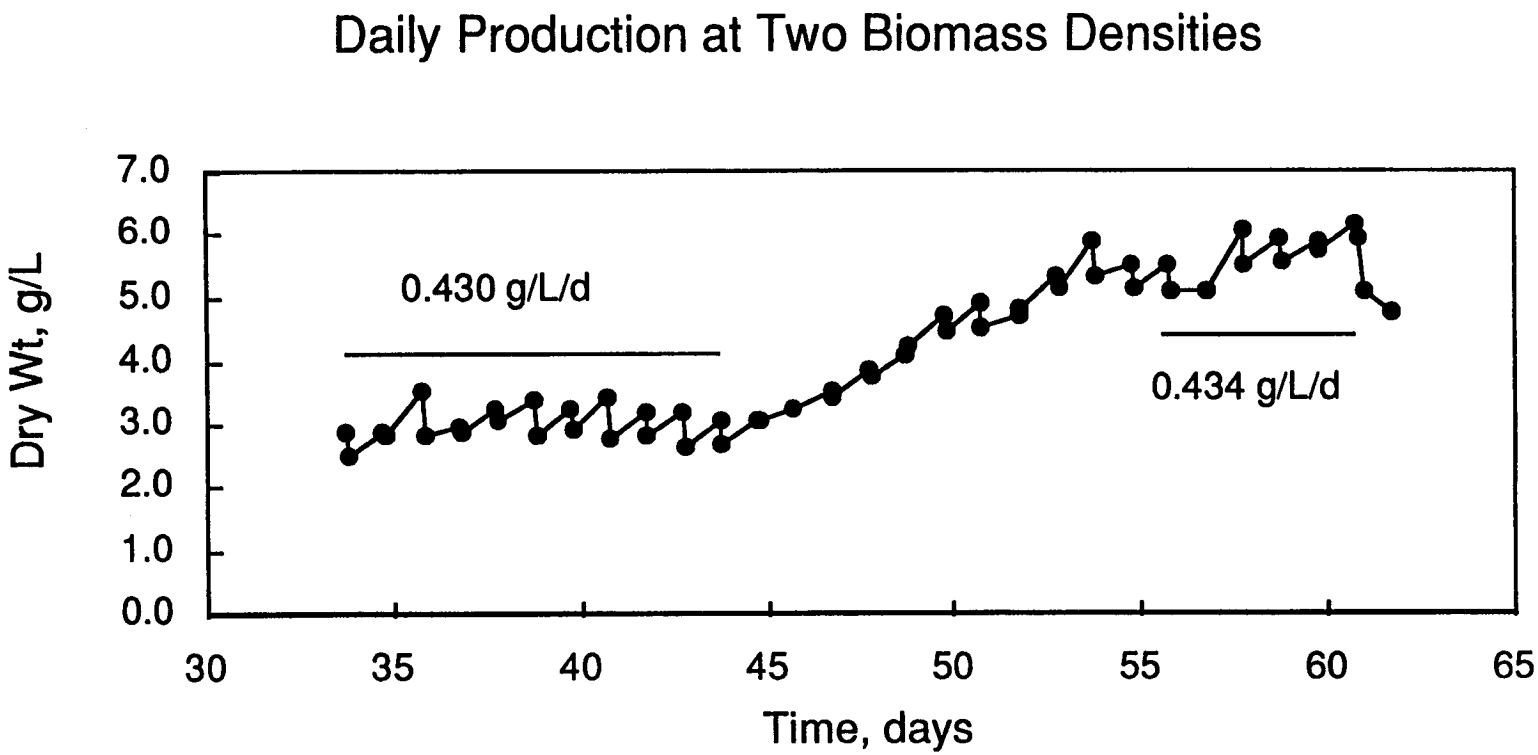
Daily Production at Two Biomass Densities
Dry Wt, g/L
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0.0
0.430 g/L/d
0.434 g/L/d
30
35
40
45
50
55
60
65
Time, days

Figure 5

Diurnal Changes in Light Intensity and Biomass

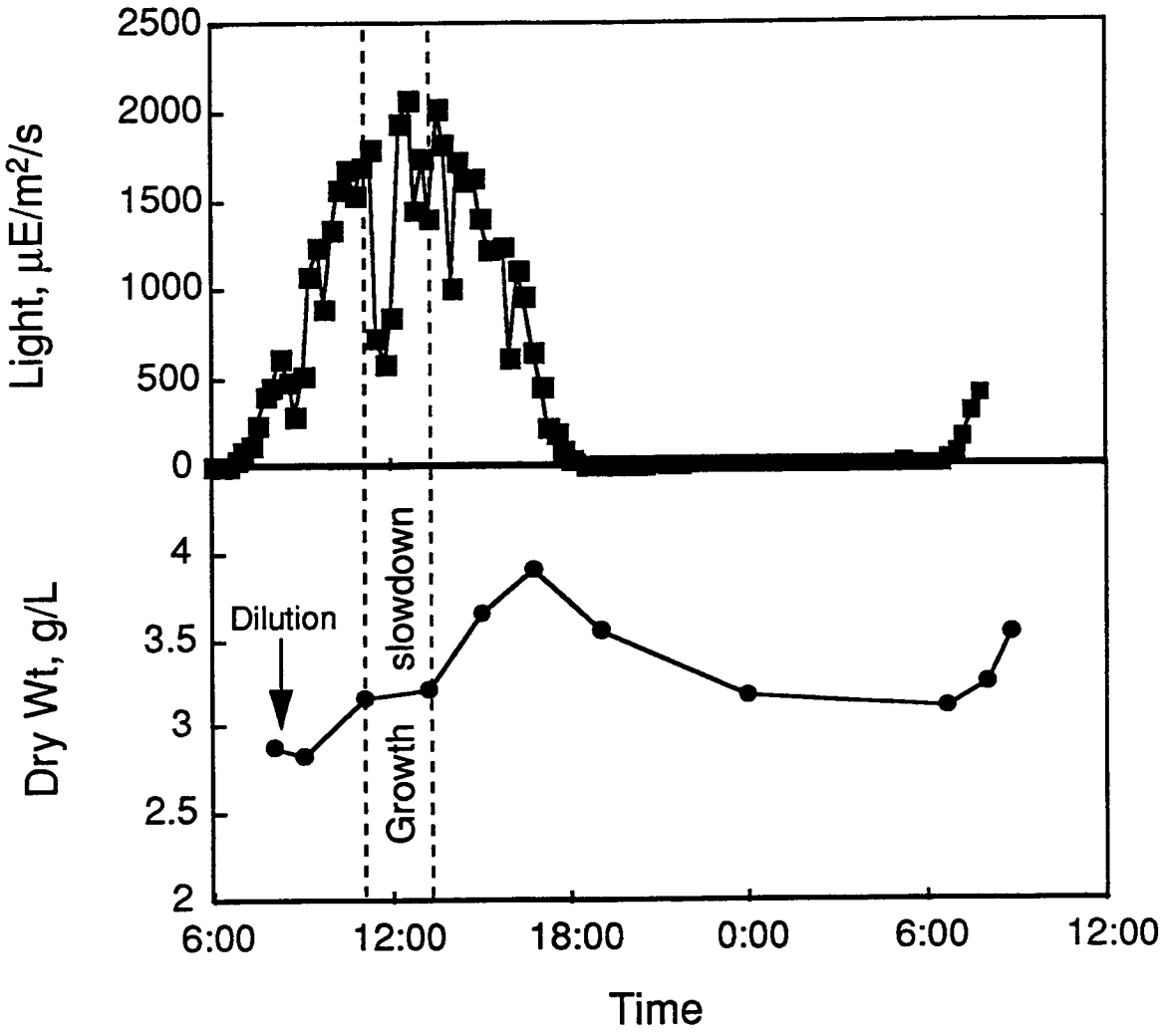

Figure 6

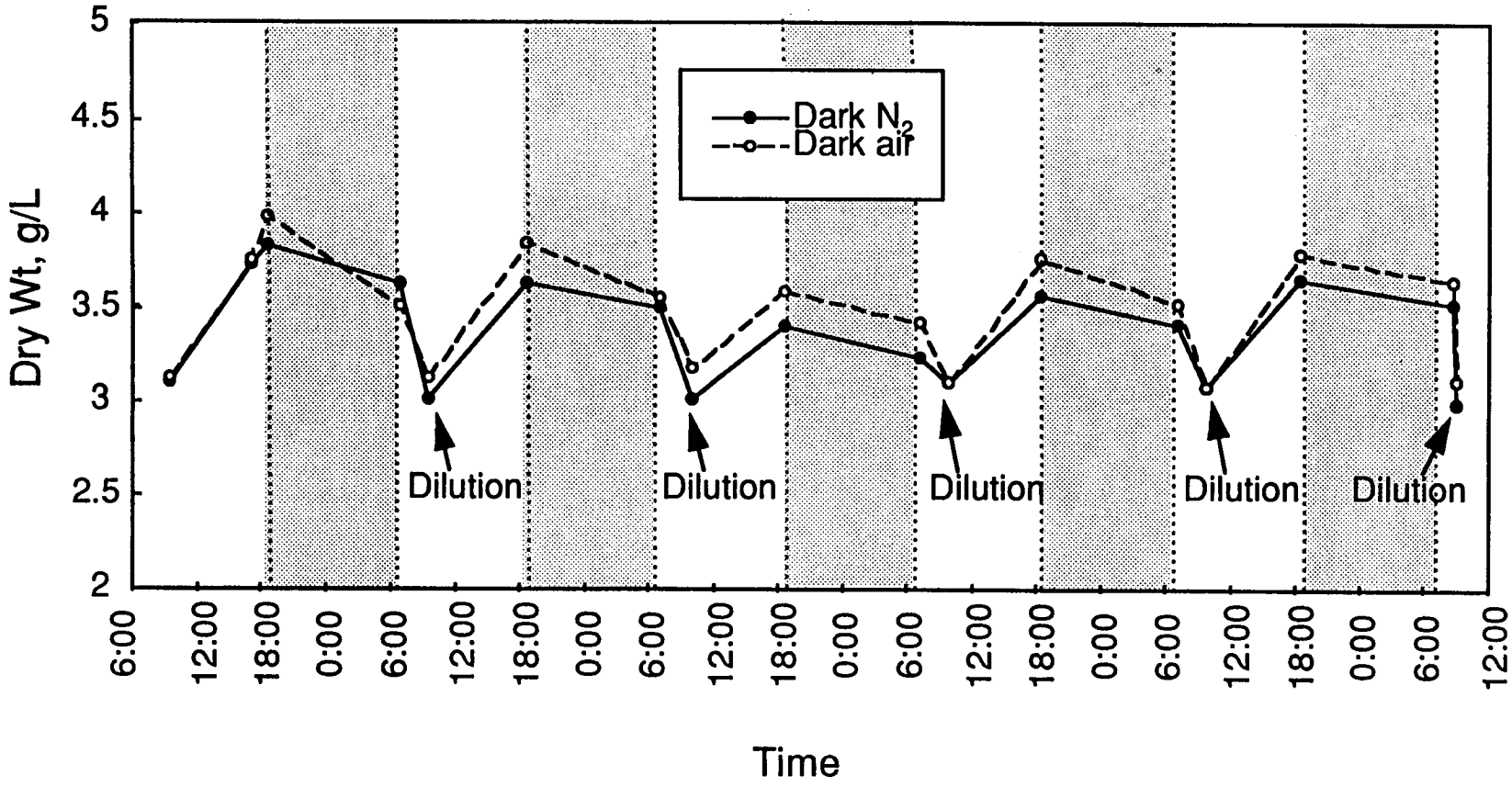
Nighttime Bubbling with N_2 vs Air
Dry Wt, g/L
5
4.5
4
3.5
3
2.5
2
Dark N_2
Dark air
Dilution
Dilution
Dilution
Dilution
Dilution
6:00
12:00
18:00
0:00
6:00
12:00
18:00
0:00
6:00
12:00
18:00
0:00
6:00
12:00
18:00
0:00
6:00
12:00
18:00
0:00
6:00
12:00
Time

Figure 7