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PHOTOSYNTHETIC WATER SPLITTING

ANNUAL REPORT

(March 1, 1985 — February 28, 1986)

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PHOTOSYNTHETIC WATER SPLITTING

ANNUAL REPORT

(March 1, 1985–February 28, 1986)

Prepared by

E. Greenbaum

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Oak Ridge, Tennessee 37831
operated by
MARTIN MARIETTA ENERGY SYSTEMS, INC.
for the
DEPARTMENT OF ENERGY
DE-AC05-84OR21400

ORNL/TM-10018

For

GAS RESEARCH INSTITUTE

Contract No. 5083-260-0880

Date Published - May 1986

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March 1986



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REPORT DOCUMENTATION PAGE	1. REPORT NO.	2.	3. Recipient's Accession No. GRI-86/0087
4. Title and Subtitle PHOTOSYNTHETIC WATER SPLITTING		5. Report Date March 1986	
7. Author(s) Elias Greenbaum		6.	
9. Performing Organization Name and Address Chemical Technology Division Oak Ridge National Laboratory Oak Ridge, Tennessee 37831		8. Performing Organization Rept. No. ORNL/TM-10018	
12. Sponsoring Organization Name and Address Gas Research Institute 8600 West Bryn Mawr Avenue Chicago, Illinois 60631		10. Project/Task/Work Unit No.	
15. Supplementary Notes		11. Contract(C) or Grant(G) No. (C) 5083-260-0880 (G)	
16. Abstract (Limit: 200 words) <p>This document is an annual report prepared for the Gas Research Institute dealing with the basic physics and chemistry of photosynthetic hydrogen and oxygen production. Two key advances have been made during the current reporting period. First, net thermodynamic efficiencies of the conversion of light energy into a gaseous fuel via photo-biological water splitting have been measured. Based on the Gibbs free energy of hydrogen production by intact microalgae, conversion efficiencies of ~10% have been measured in the linear, low-intensity region of the light saturation curve of photo-synthesis. These are, by far, the highest conversion efficiencies reported for photo-biological hydrogen production. Success in this measurement was based, in part, on a recognition of the physiological state of the algae with respect to the number of monolayers used for the measurement. Second, a key advance in biotechnology methodology has been made in the area of rapid assay and screening techniques for gaseous fuel synthesis of microalgae. Working with isolated colonies cultured on specially formulated, optically black, solid-growth media, hydrogen production by individual colonies of algae irradiated with an X-Y translatable helium-neon laser has been quantitatively measured. It is believed that this newly developed methodology represents an important instrumental advance for applying modern techniques of genetics and molecular biology to the problem of gaseous fuel synthesis from renewable inorganic resources via microalgal water splitting.</p>		13. Type of Report & Period Covered 14. Annual Report Mar. 1, 1985-Feb. 28, 1986	
17. Document Analysis a. Descriptors b. Identifiers/Open-Ended Terms c. COSATI Field/Group			
18. Availability Statement: National Technical Information Service U.S. Department of Commerce 5825 Port Royal Road; Springfield, VA 22161		19. Security Class (This Report) Unclassified	21. No. of Pages
		20. Security Class (This Page) Unclassified	22. Price

RESEARCH SUMMARY

Title	Photosynthetic Water Splitting
Contractor	Oak Ridge National Laboratory
	GRI Contract Number: 5083-260-0880
Principal Investigator	E. Greenbaum
Report Period	March 1, 1985 -- February 28, 1986 Annual Report
Objective	To understand the basic physics and chemistry of hydrogen and oxygen photoproduction by the photosynthetic process.
Technical Perspective	Photosynthetic water splitting is a promising approach to the production of hydrogen as a renewable gaseous fuel. In essence, a biological solar energy conversion and storage system is contemplated in which the energy-rich product is molecular hydrogen. Hydrogen has potential applications as a possible addition to or substitute for natural gas.
Results	Initial success in this mission-oriented basic research program was achieved by developing physical instrumentation for performing kinetic and mechanistic studies of the simultaneous photoproduction of molecular hydrogen and oxygen by anaerobically adapted green algae. Two key advances have been made during the current reporting period. First, net thermodynamic efficiencies of the conversion of light energy into a gaseous fuel via photobiological water splitting have been measured. Based on the Gibbs free energy of hydrogen production by intact microalgae, conversion efficiencies of ~10% have been measured in the linear, low-intensity region of the light saturation curve of photosynthesis. These are, by far, the highest conversion efficiencies reported for photobiological hydrogen production. Success in this measurement was based, in part, on a recognition of the physiological state of the algae with respect to the number of monolayers used for the measurement. Second, a key advance in biotechnology methodology has been made in the area of rapid assay and screening techniques for gaseous fuel synthesis of microalgae. Working with isolated colonies cultured on specially formulated, optically black, solid-growth media, hydrogen production by individual colonies of algae irradiated with an X-Y translatable helium-neon laser has been quantitatively measured. It is believed that this newly developed

Results (contd.)	methodology represents an important instrumental advance for applying modern techniques of genetics and molecular biology to the problem of gaseous fuel synthesis from renewable inorganic resources via microalgal water splitting.
Technical Approach	These results were obtained by developing a novel apparatus for measuring the simultaneous photoproduction of hydrogen and oxygen. The apparatus consists of a flow system in which the hydrogen and oxygen sensors are located downstream from the photosynthetic reactor. Physical removal of the sensors from the locus of gas production has the important technical advantage of eliminating interfering light-induced artifacts in the sensors. The hydrogen sensor is a Taguchi gas-sensitive semiconductor. The oxygen sensor is a Hersch electrogalvanic cell. Using an electrolysis cell and Faraday's Law of Electrochemical Equivalence, calibration curves for the two sensors are prepared using a least-squares fitting routine in conjunction with an HP-85 laboratory microcomputer.
Project Implications	During the previous year this research has continued to advance the possibility of effective biophotolytic production of H ₂ and O ₂ from water. Although obtained under special, low intensity, anaerobic, laboratory conditions, the large improvement over previously reported efficiencies indicates that living photosynthetic systems have potential for splitting water efficiently. The laser method for rapid assay of H ₂ produced from isolated colonies is the subject of a GRI patent application and will expand the application of genetic and molecular biology techniques to the research. During the following year, the research will emphasize efforts to improve performance at higher light intensity. GRI plans to continue support of this research.

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1.0 ANNUAL REPORT

The following sections describe experimental research on gaseous fuel production from inorganic resources based on photosynthetic water splitting by microalgae for hydrogen and oxygen production. Each section is divided into three parts: Rationale, Results, and Status.

The Rationale section describes why we are performing this particular task and how it contributes to GRI's strategy of renewable gaseous fuel production. This section also highlights the scientific merit of the research in the specific context of the contract.

The Results section is a technical description of experimental results and, where appropriate, a description of the experimental protocol used to obtain those results.

The Status section indicates the extent of completion of this task and, if completed, its significance in the context of GRI objectives.

Photosynthetic water splitting by microalgae for the production of hydrogen and oxygen is a promising approach to the production of gaseous fuels from renewable inorganic resources. The mission-oriented basic research performed in GRI's Photosynthetic Water Splitting research project is focused on a quantitative assessment of a biotechnological process for gaseous fuel synthesis from renewable inorganic resources using living microalgae. Microalgal photosynthesis is a logical basic research area for the production of gaseous fuel from inorganic resources because the algae themselves are synthesized in a self-replicating process requiring only water, mineral salts, visible light, and carbon dioxide as the sole carbon source. Moreover, as was first shown by Gaffron and Rubin (1), certain microalgae possess a unique biochemistry. A specialized enzyme, hydrogenase, can catalyze the photoevolution of the gaseous

fuel hydrogen. As documented in previous GRI Annual Reports and peer-reviewed professional publications (2-12), we have demonstrated that certain unicellular microalgae such as *Chlamydomonas* and *Scenedesmus* are capable of the simultaneous photoevolution of hydrogen and oxygen for hundreds of hours. We have also demonstrated the potential for selecting and improving subcultures of algae with enhanced properties for hydrogen and oxygen evolution (8,10).

Research during the current reporting period has focused on two key areas: (a) measurement of net thermodynamic conversion efficiencies and (b) original instrumentation development for rapid analysis of photo-produced hydrogen by isolated algal colonies.

1.1 Energy Conversion Efficiencies

Rationale. The most important figure of merit for any photo-conversion concept is net thermodynamic conversion efficiency. As used in this report, efficiency is defined as the ratio of Gibbs free energy of photoproducted molecular hydrogen to solar-equivalent absorbed radiation. The photosynthetically active radiation was assumed to comprise half of the power of the solar emission spectrum; therefore, the solar-equivalent efficiency was determined by multiplying the photosynthetically active efficiency by two.

Results. The results of the efficiency experiments are indicated in Table 1. Figure 1 is a schematic illustration of the reaction chamber used. The dotted circle in the center represents a relatively thin film of unicellular algae (<30 monolayers) entrapped on filter paper. The algae are centered on and pressed flush against an aluminum backing plate that has a hole which is bored concentric with the circle defined

by the entrapped algae. This hole was able to accommodate the sensing head of an EG&G Model 550 radiometer equipped with a flat spectral filter. Irradiation of the algae was perpendicular to the plane defined by the filter paper and the retaining ring. The algae were covered by an O-ring plate with a flat window, which provided a uniformly clear pathway for illumination. This cover plate and the associated backing support yielded a hermetically sealed environment which was placed serially in a flow system, as indicated by the longitudinal O-ring connectors of Fig. 1. Hydrogen and oxygen produced in the algae diffused into the adjacent gas phase and were swept out of the reaction chamber and carried downstream to separate hydrogen and oxygen sensors.

The methodology for efficiency measurements is further illustrated in Fig. 2. Figure 3 contains data from which efficiencies were determined. As indicated in Fig. 2, each conversion efficiency measurement was based on two light measurements, one with algae-entrapped filter paper and another with the filter paper alone. Efficiencies were computed based on net absorbed photosynthetically active radiation and then divided by two to obtain an equivalent solar efficiency.

As can be seen in Table 1, the equivalent solar efficiencies vary from 3 to 12%, depending on the alga and the irradiation interval.

Status. The conversion efficiencies indicated in Table 1 are the highest values ever reported for photobiological hydrogen production and, in our opinion, are an encouraging development in the area of hydrogen fuel synthesis via photosynthetic water splitting. However, it is important to remember that the result applies only in the linear low-intensity region of the light saturation curve. A kinetic imbalance between photon absorption and thermally activated electron transport is a well-known

characteristic of photosynthesis. The question of net conversion efficiency and its relationship to incident irradiance is among the most important tasks of this research project. It is an ongoing item and will be further discussed in Sect. 2.0.

1.2 Light-Scattering Measurements

Rationale. Optical measurements on heterogenous systems such as microalgae are complicated by light scattering. Since conversion efficiencies involve an absolute light measurement, the magnitude and angular distribution of scattered light are important factors. Although a complete understanding of light scattering of heterogenous media involves complex theoretical treatment, its effect on the calculated efficiency is easily evaluated. In the efficiency calculation, any light that does not reach the photodetector (i.e., backscattered light) is counted as being absorbed and goes into the denominator of the efficiency computation. Because this backscattered light is not involved in photobiochemistry, the net effect is to underestimate the actual efficiency. However, the effect is minimal for the algae used in the current experiments since most of the scattered light is forward scattered in a small angle cone about the axis of irradiation (see Results section).

Results. The light-scattering measurements were performed by M. T. Harris, in association with C. H. Byers and D. F. Williams, at Oak Ridge National Laboratory (ORNL). Figure 4 is a schematic illustration of the laser-light-scattering facility used for these measurements. The sample cell was replaced by two thin quartz plates between which the algae were sandwiched. The algae were illuminated with the 514.5-nm line of an argon-ion laser. Two runs were performed. Plots of scattered

light vs angular position are presented in Figs. 5 and 6. The detector radius was 47 cm. The planar angle subtended by the diameter of the detector was 1.4° for the data of Fig. 5 and 0.4° for the data of Fig. 6.

The key conclusion of these experiments is that virtually all of the light that is scattered by the alga is in a small-angle forward cone, which is subtended by the solid angle of the radiometric sensor.

Status. This task is complete.

1.3 Biotechnological Instrumentation Development

Rationale. New instrumentation has been developed utilizing an *in situ* rapid assaying and screening technique for the photoproduction of hydrogen from individual, isolated colonies of microalgae.

With current instrumentation, the screening of an algal population for hydrogen photoproduction is time-consuming and labor intensive. In addition, we have discovered and reported (8,10) that a considerable amount of variability, with respect to both hydrogen production and survivability for extended periods of anaerobiosis, exists among the individual cells of a pure-strain algal culture. The new method being tested will allow a rapid assay of the hydrogen-producing capacities of a large number of algal colonies (a colony is defined as a population of cells, all of which are descended from a single cell by vegetative reproduction).

In addition to providing a means for screening colonies rapidly, this new technique will allow quantitation of the variability in hydrogenase activity seen in large populations of algae.

The method is based on the technique of spreading dilute algal suspensions over the surface of agar-solidified medium, which produces

well-spaced colonies over the surface of the agar after a sufficient growth period (~2 weeks). Each of these colonies of ~1 to 2 mm in diameter contains from 10^5 to 10^6 cells, and each cell is a direct lineal descendant of the parent cell. This ensures (barring spontaneous mutation) that all the cells in a particular colony are genetically homogeneous; as such, each cell in the colony should have an identical capacity for hydrogen photoproduction. However, since heterogeneity exists among cells in liquid cultures with respect to hydrogen production capabilities, this same variability should be evident among the individual colonies on the agar plate.

These agar plates are placed in a reactor modified to fit their dimensions. This reactor is incorporated into the previously described flow-through analysis system, with downstream monitors for oxygen and hydrogen. The individual colonies on the plates are illuminated by a helium-neon laser, which allows a single colony to be illuminated while the other colonies remain in darkness. A potential problem is laser-light scattering by the agar, the algal colony, and the plastic Petri dish, which can illuminate other colonies on the plate. However, the addition of fine-mesh charcoal to the solid medium has solved this problem.

Our key objectives in pursuing this research are:

- a. to provide a rapid, simple, and sensitive assay of hydrogenase activity in green algae to facilitate the selection and maintenance of clones with elevated rates of hydrogen production;
- b. to more easily and accurately quantify the variability among cells in ostensibly pure-strain algal cultures with respect to hydrogen photoproduction capabilities;

- c. to screen large numbers of algal species and strains and to select from those populations clones of algae with extraordinarily high rates of hydrogen photoproduction which could possibly raise the efficiency levels of reactors for photobiological hydrogen production.

Results. Table 2 gives representative rates of hydrogen production from single colonies of wild-type strains of *Chlamydomonas reinhardtii*. All of the colonies in Table 2 were approximately 3 weeks old and 1 to 3 mm in diameter.

Status. This task is in an early stage and will be continued in the next contract year.

2.0 FUTURE RESEARCH

The research planned for next year follows logically from current accomplishments of this project and has a specific mission-oriented goal — the demonstration of sustained high-efficiency gaseous fuel production via photosynthetic water splitting by microalgae. Accordingly, two areas of research will be the principal points of focus for the coming year.

First, we will build on the promising results that demonstrate high conversion efficiencies in the linear low-intensity region of the light-saturation curve. One question that we must resolve is whether these high efficiencies can be sustained at higher incident light intensities. We believe that the answer is yes and that the key to this problem is the utilization of mutant algae with relatively small optical cross sections for the water-splitting reaction, in comparison to wild-type algae. Toward this end, we are collaborating with geneticists and physiologists, in particular Professor Gregory W. Schmidt, of the Botany Department,

University of Georgia, who has provided us with two low-chlorophyll mutant strains of *Chlamydomonas* for study.

Second, we will use our laser screening technology to survey individual clones of microalgae for enhanced properties of light-induced gaseous fuel synthesis. In addition to its obvious practical implications, we believe that this work will make a significant contribution in the basic research area of hydrogenase and algal genetics.

3.0 PUBLICATIONS

The following publications were based on GRI-supported research:

1. E. Greenbaum, "Hydrogen Production by Algal Water Splitting," to be published as a chapter in *Algae and Human Affairs*, C. A. Lembi and J. R. Waaland (Eds.), Cambridge University Press (1986).
2. E. Greenbaum, "Photosynthetic Water Splitting: A Biotechnological Approach to Gaseous Fuel Synthesis," in *Proceedings of the 1986 International Gas Research Conference*, September 8-11, 1986, Toronto, Canada (in press).
3. H. B. Ward, M. E. Reeves, and E. Greenbaum, "Stress-Selected *Chlamydomonas reinhardtii* for Photoproduction of Hydrogen," *Biotechnol. Bioeng. Symp.*, in press (1986).
4. E. Greenbaum, "Hydrogen and Oxygen Production by Photosynthetic Water Splitting," in *Proceedings of the Second International Symposium on Hydrogen Produced from Renewable Energy*, O. G. Hancock and K. G. Sheinkopf (Eds.), pp. 145-53 (1985).
5. M. E. Reeves and E. Greenbaum, "Long-Term Endurance and Selection Studies in Hydrogen and Oxygen Photoproduction by *Chlamydomonas reinhardtii*," *Enzyme and Microbial Technol.* 7, 169-74 (1985).

4.0 SEMINARS AND INVITED PAPERS

The following seminars and invited papers were presented during the current reporting period:

1. M. E. Reeves and E. Greenbaum, "Photosynthetic Unit Sizes for Hydrogen and Oxygen Photoproduction in Intact Algae," poster presentation at the Seventh Symposium on Biotechnology for Fuels and Chemicals, May 14-17, 1985, Gatlinburg, TN.
2. H. B. Ward, M. E. Reeves, and E. Greenbaum, "Stress-Selected *Chlamydomonas reinhardtii* for Photoproduction of Hydrogen," poster presentation at the Seventh Symposium on Biotechnology for Fuels and Chemicals, May 14-17, 1985, Gatlinburg, TN.
3. B. Ward, M. E. Reeves, and E. Greenbaum, "Anaerobiosis Tolerance and Suitability of *Chlamydomonas reinhardtii* Strains for Long-Term Production of Hydrogen," Second International Phycological Congress, August 4-10, 1985, Copenhagen, Denmark.
4. E. Greenbaum, "The Light Saturation Curves of Photosynthetic Hydrogen and Oxygen Evolution," presented at the Annual Meeting of the American Society for Photobiology, June 23-27, 1985, New Orleans, LA.
5. M. Reeves and E. Greenbaum, "Photosynthetic Unit Sizes for Hydrogen and Oxygen Photoproduction in Wild-Type and Mutant Strains of *Chlamydomonas reinhardtii*," Second Conference on the Genetics and Molecular Biology of *Chlamydomonas*, August 11-15, 1985, Santa Cruz, CA.
6. E. Greenbaum, "Physics and Chemistry of Biological Water Splitting," paper presented to the Department of Applied Biology, Georgia Institute of Technology, March 10, 1986.
7. E. Greenbaum, "Energetic Aspects of Photosynthetic Water Splitting," Symposium on the Structural and Energetic Aspects of Light-Induced Water Splitting General Meeting of the American Physical Society, March 31-April 4, 1986, Las Vegas, NV.
8. E. Greenbaum, "Physics and Chemistry of Biological Water Splitting," paper presented to the Duke University Marine Laboratory, April 15, 1986, Beaufort, NC.

5.0 SERVICE TO PROFESSIONAL SOCIETIES AND COLLABORATIVE RESEARCH

5.1 Professional Societies

The principal investigator is a member of the Executive Committee of the Division of Biological Physics of the American Physical Society and has been elected Secretary-Treasurer of the Division. He is a member of the Board of Directors and Past-Chairman of the Division of Biotechnology

and Chemical Sciences of the American Solar Energy Society. He is also organizer and chairperson of the Special Topic Discussion Groups at the Eighth Symposium on Biotechnology for Fuels and Chemicals, May 13-16, 1986, Gatlinburg, TN.

5.2 Collaborative Research

Professor Gregory W. Schmidt, of the Botany Department, University of Georgia, has isolated mutants of *Chlamydomonas* with low chlorophyll content *and* the ability to grow exclusively on inorganic substrates, using carbon dioxide as the sole carbon source. This class of mutants is of potential interest with respect to the relative size of the optical cross section for the water-splitting reaction. We plan to collaborate with Professor Schmidt in characterizing the light-response properties of these and other algae.

In addition, we are continuing our collaborative research with Dr. R. R. L. Guillard, of the Bigelow Laboratory for Ocean Sciences, West Boothbay Harbor, Maine, on the utilization of microscopic marine algae for hydrogen and oxygen production.

6.0 SPECIAL RECOGNITION

Based in part on GRI-funded research, the principal investigator has been nominated for Fellowship in the American Association for the Advancement of Science. He received a 1986 Technical Communication Award, presented by the East Tennessee Chapter of the Society for Technical Communication, and has also received a Distinguished Scientist Award, presented by the Martin Marietta Corporation.

7.0 REFERENCES

1. H. Gaffron and J. Rubin, "Fermentative and Photochemical Production of Hydrogen in Algae," *J. Gen. Physiol.* **26**, 219-40 (1942).
2. E. Greenbaum, "Application of Intact Algae to the Biophotolysis Problem," *Biotechnol. Bioeng. Symp.* **12**, 469-74 (1982).
3. E. Greenbaum and J. Ramus, "Survey of Selected Seaweeds for the Simultaneous Photoproduction of Hydrogen and Oxygen," *J. Phycol.* **19**, 53-57 (1983).
4. E. Greenbaum, R. R. L. Guillard, and W. G. Sunda, "Hydrogen and Oxygen Photoproduction by Marine Algae," *Photochem. Photobiol.* **37**, 649-55 (1983).
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8. M. E. Reeves and E. Greenbaum, "Long-Term Endurance and Selection Studies in Hydrogen and Oxygen Photoproduction by *Chlamydomonas reinhardtii*," *Enzyme and Microbial Technol.* **7**, 169-74 (1985).
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12. E. Greenbaum, "Hydrogen Production by Algal Water Splitting," to be published as a chapter in *Algae and Human Affairs*, C. A. Lembi and J. R. Waaland (Eds.), Cambridge University Press (1986).

Table 1. Energy conversion efficiencies of green algae for hydrogen and oxygen production

Experiment no.	Alga	Irradiation interval ^a	Efficiency (PAR) (%) ^b	Equiv. solar eff. (%) ^c
1	<i>Scenedesmus</i> D ₃	1	16	8
		2	23	11.5
2	<i>Chlamydomonas reinhardtii</i> (sup)	1	13	6.5
		2	16	8
		3	18	9
		4	19	9.5
		5	21	10.5
		6	21	10.5
		7	18	9
3	<i>Chlamydomonas reinhardtii</i> (UTEX 90)	1	6	3
		2	8	4
		3	8	4
4	<i>Chlamydomonas moewusii</i>	1	24	12
		2	22	11
		3	18	9

^aThe irradiation intervals consisted of sequential cycles of 3 h on, 3 h off.

^bConversion efficiency is based on absorbed photosynthetically active radiation.

^cColumn 2 is divided by 2, assuming that the photosynthetically active radiation comprises half of the power in the solar emission spectrum.

Table 2. Rates of hydrogen photoproduction from single colonies of *Chlamydomonas reinhardtii*^a

Experiment no.	Algal strain	Maximum rate of H ₂ photoproduction (nmol/h)
1	UTEX #2246	16
2	CC-125 [137c(+)]	7
3	UTEX #2246	16
4	UTEX #2246	26
5	CC-125	21
6	CC-125	9

^aThese absolute rates are not normalized to colony diameter or chlorophyll content.

8.0 FIGURE LEGENDS

Fig. 1. Schematic illustration of reaction chamber used to measure thermodynamic conversion efficiencies.

Fig. 2. Principle of conversion efficiency measurements based on net light absorption by thin films of algae.

Fig. 3. Simultaneous photoproduction of hydrogen and oxygen by entrapped *Chlamydomonas moewusii*.

Fig. 4. Schematic illustration of laser-light-scattering facility used to determine angular distribution of scattered light from algae.

Fig. 5. Angular distribution data used to determine scattered light from algae.

Fig. 6. Same as Fig. 5, but a smaller angle is subtended by the photodetector. See text for details.

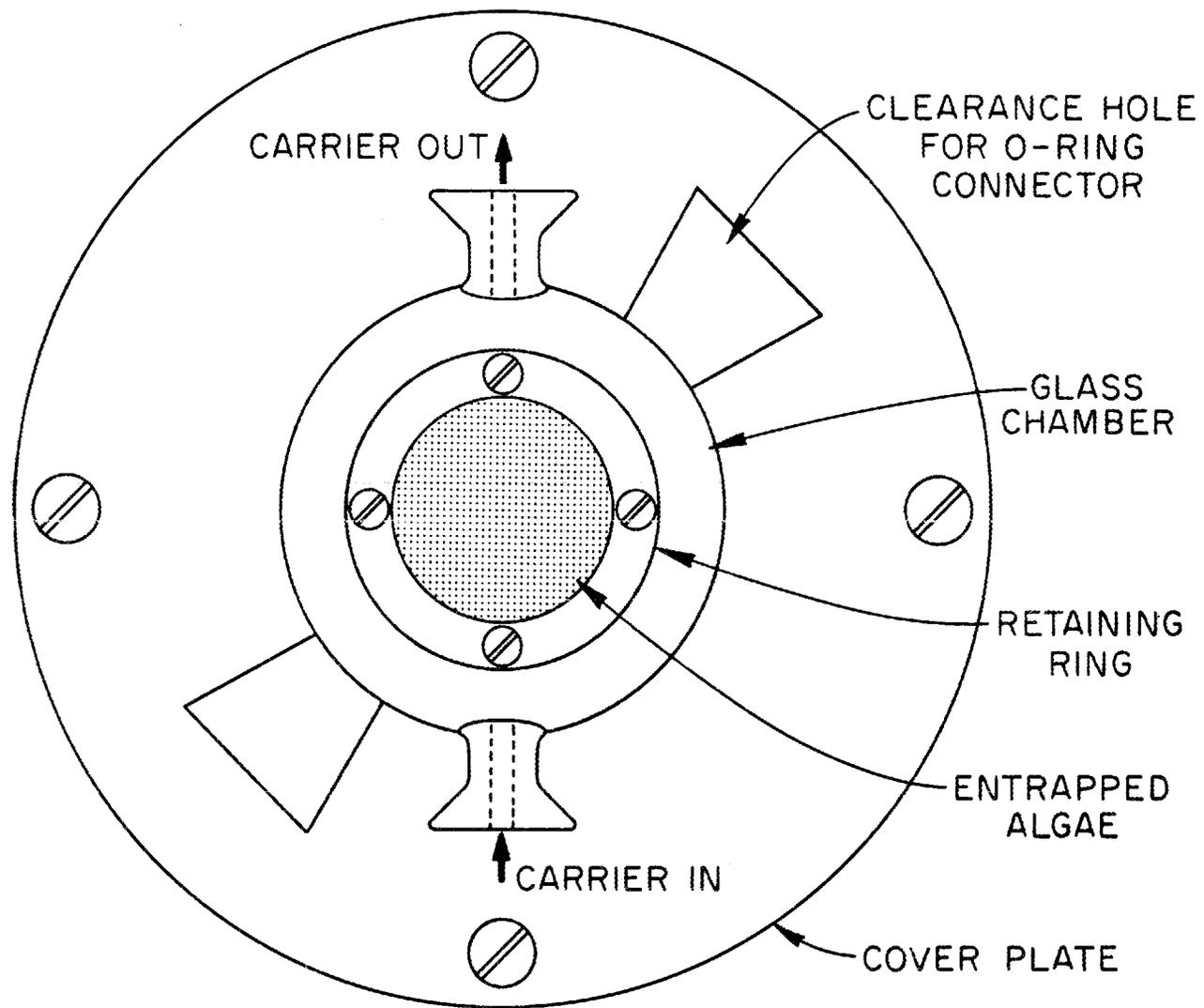


Figure 1

EFFICIENCY MEASUREMENTS

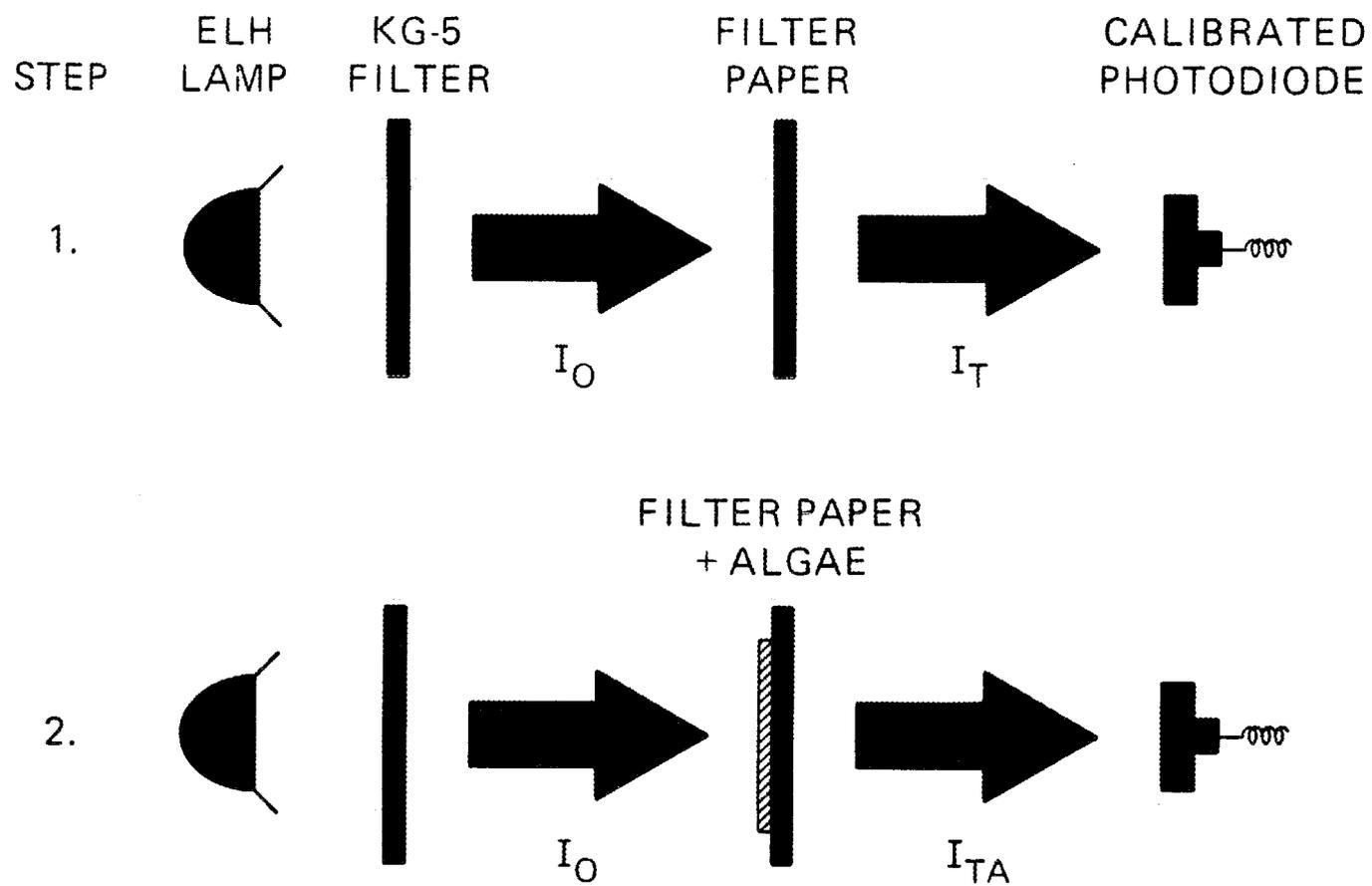
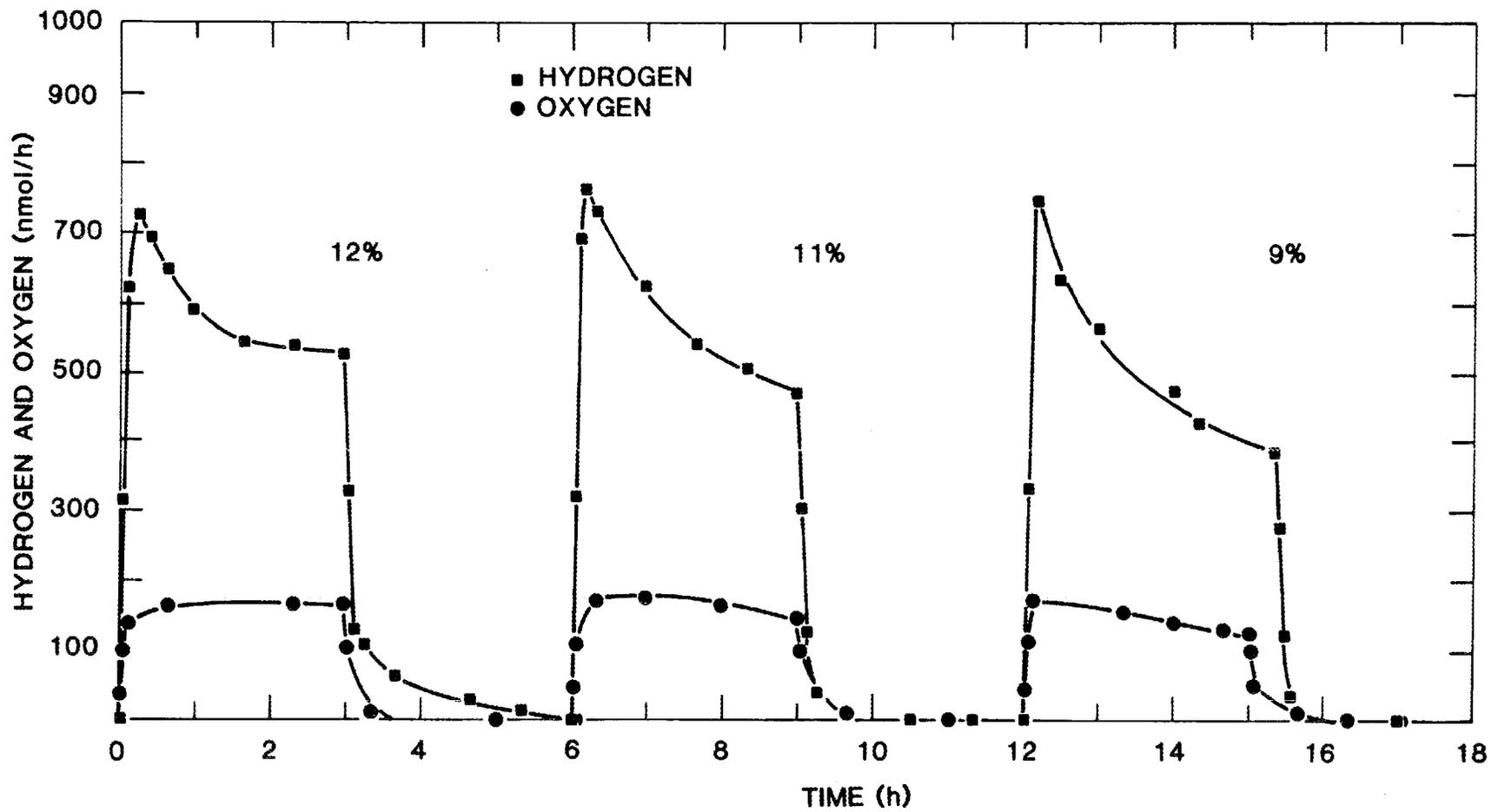
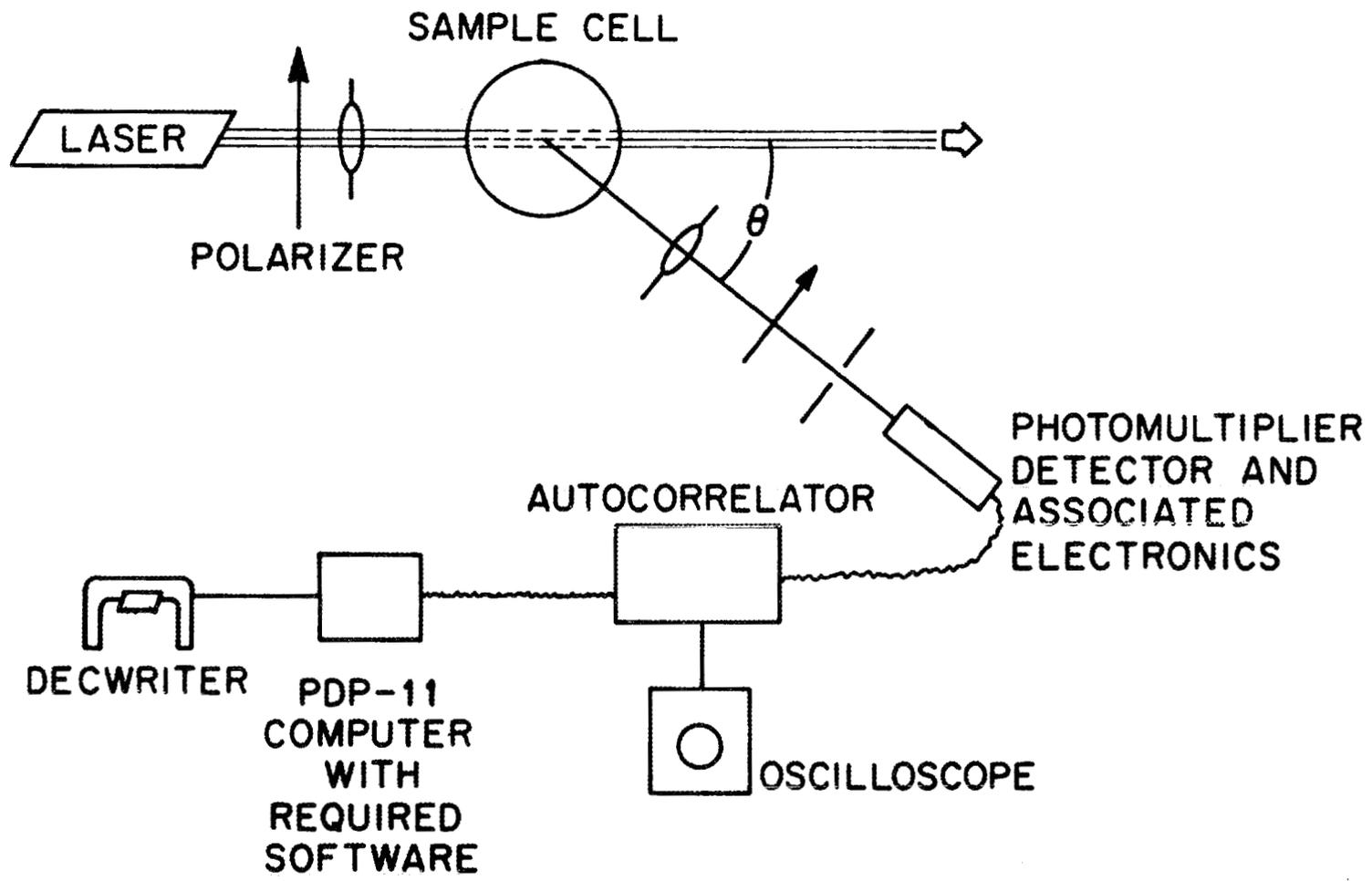


Figure 2



**SIMULTANEOUS PHOTOEVOLUTION OF HYDROGEN AND OXYGEN
BY *Chlamydomonas moewusii***

Figure 3



PROPOSED LASER LIGHT SCATTERING FACILITY

Figure 4

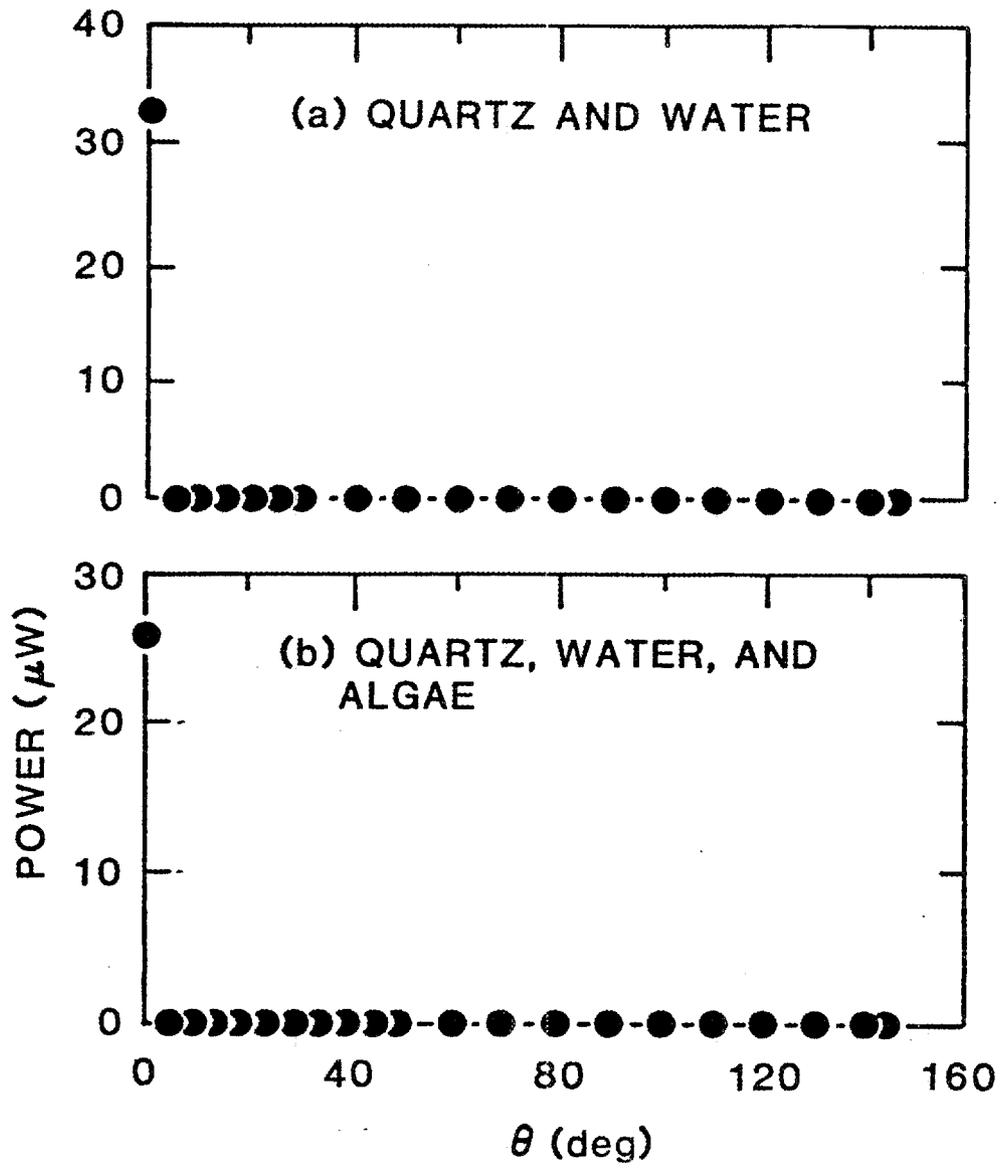


Figure 5

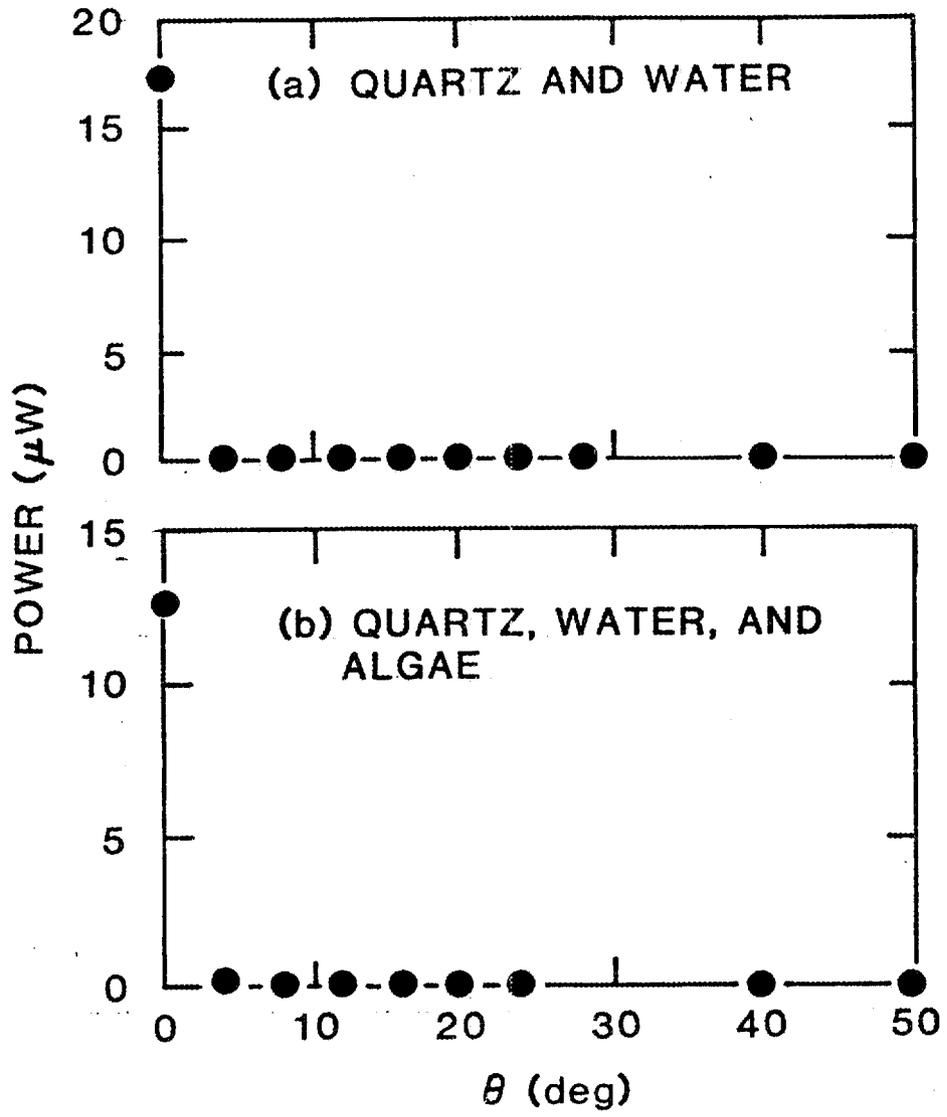


Figure 6

