APPENDIX C

National Science Foundation
Small Business Innovation Research Program

PROJECT SUMMARY

NSF AWARD NO.

NAME OF FIRM	Protein Solutions, Inc. (PSI)
ADDRESS	350 West 800 North, Suite 218 Salt Lake City, Utah 84103
PRINICPAL INVESTIGATOR (NAME AND TITLE)	Robert Scheer, Ph.D.
TITLE OF PROJECT	Marine Phytoplankton in Sealed Environments
TOPIC TITLE: Ocean Studies	TOPIC NUMBER AND SUBTOPIC LETTER: 8b

This Phase I SBIR project is on marine phytoplankton.

Certain marine phytoplankton can be maintained in culture in sealed environments for extended periods of time. We propose to study the characteristics of cultures maintained in sealed, transparent, gas semi-permeable "plastic" bags. The ability of phytoplankton to grow in such sealed environments opens a wide range of possible applications, including the development of living specimens for science education, detectors and sensors for various agents, controlled quantitites ofliving plankton as food for other organisms, and stock maintenance for archival and research purposes. Most laboratory and large scale cultures of phyto-plankton are open systems. There is little literature on the growth of these organisms insealed or closed environments.

Bioluminescent dinoflagellates are of particular interest as the light emission process is an indicator of the health and numbers of organisms.

We propose to perform fundamental studies on the growth, longevity, and stability of bioluminescent phytoplankton cultures in sealed but partially gas permeable environments. The goal is to understand the photosynthesis/respiration ratios, and the effect of pH, PO_2, PCO_2, as well as O_2, CO_2, H_2S, and CH_4 in the gas phase. These parameters will be studied as a function of temperature, lighting conditions, culture medium, and surface to volume ratios. These studies should have wide applicability to the development of sealed phytoplankton cultures for education purposes, as food for marine organisms, as a means to facilitate the transport of stocks from site to site, and as a simple means of producing phytoplankton for biotechnol-ogical studies.

Potential Commercial Applications of the Research Materials and Methods for producing phytoplankton for:

1) the pharmaceutical and biotechnology industries;
2) the home hobby aquarium market;
3) the display aquarium market;
4) science education;
5) as detectors and sensors for toxic agents;
6) food for other aquaculture applications.

KEY WORDS TO IDENTIFY RESEARCH OR TECHNOLOGY (8 MAXIMUM)
Phytoplankton, Bioluminescence, Photosynthesis, Respiration, Aquaculture, Ecosystem, Mesocosm, Education.

APPENDIX B (continued)

CERTIFICATION PAGE

Certification for Principal Investigators and Co-Principal Investigators:

I certify to the best of my knowledge that:
(1) the statements herein (excluding scientific hypotheses and scientific opinions) are true and complete, and
(2) the text and graphics herein as well as any accompanying publications or other documents, unless otherwise indicated, are the original work of the signatories or individuals working under their supervision. I agree to accept responsibility for the scientific conduct of the project and to provide the required progress reports if an award is made as a result of this application.

I understand that the willful provision of false information or concealing a material fact in this proposal or any other communication submitted to NSF is a criminal offense (U.S. Code, Title 18, Section 1001).

Name (Typed)	Signature	Date
PI/PD R. Scheer	Robert J. Scheer	6/10/94
Co-PI/PD		
Co-PI/PD		
Co-PI/PD		

Certification for Authorized Company Representative

By signing and submitting this proposal, the individual applicant or the authorized official of the applicant institution is: (1) certifying that statements made herein are true and complete to the best of his/her knowledge; and (2) agreeing to accept the obligation to comply with NSF award terms and conditions if an award is made as a result of this application. Further, the applicant is hereby providing certifications regarding Federal debt status, debarment and suspension, drug-free workplace, and lobbying activities (see below), as set forth in Grant Proposal Guide (GPM), NSF 94-02. Willful provision of false information in this application and its supporting documents or in reports required under an ensuing award is a criminal offense (U.S. Code, Title 18, Section 1001).

Debt and Debarment Certification (If answer "yes" to either, please provide explanation.)

Is the organization delinquent on any Federal debt? Yes ___ No__
Is the organization or its principals presently debarred, suspended, proposed for debarment, declared ineligible, or voluntarily excluded from covered transactions by any Federal department of agency? Yes ___ No__

Certification Regarding Lobbying

This certification is required for an award of a Federal contract, grant, or cooperative agreement exceeding $100, 000 and for an award of a Federal loan of a commitment providing for the United States to insure or guarantee a loan exceeding $150,000.

Certification for Contracts, Grants, Loans and Cooperative Agreements

The undersigned certifies, to the best of his or her knowledge and belief, that:

(1) No federal appropriated funds have been paid or will be paid, by or on behalf of the undersigned, to any person for influencing or attempting to influence an officer or employee of any agency, a Member of Congress, an officer or employee of Congress, or an employee of a Member of Congress in connection with the awarding of any federal contract, the making of any Federal grant, the making of any Federal loan, the entering into of any cooperative agreement, and the extension, continuation, renewal, amendment, or modification of any Federal contract, grant, loan, or cooperative agreement.
(2) If any funds other than Federal appropriated funds have been paid or will be paid to any person for influencing or attempting to influence an officer or employee of any agency, a Member of Congress, an officer or employee of Congress or any employee of a Member of Congress in connection with this Federal contract, grant, loan, or cooperative agreement, the undersigned shall complete and submit Standard Form LLL, "Disclosure Form to Report Lobbying," in accordance with its instructions.
(3) The undersigned shall require that the language of this certification be included in the award documents for all subawards at all tiers including subcontracts, subgrants, and contracts under grants, loans, and cooperative agreements and that all subrecipients shall certify and disclose accordingly.

This certification is a material representation of fact upon which reliance was placed when this transaction was made or entered into. Submission of this certification is a prerequisite for making or entering into this transaction imposed by section 1352, title 31, U.S. Code. Any person who fails to file the required certification shall be subject to a civil penalty of not less than $10,000 and not more than $100,000 for each such failure.

AUTHORIZED COMPANY REPRESENTATIVE	SIGNATURE	DATE
NAME/TITLE (TYPED) J. ANDRADE / President		6/11/94
TELEPHONE NUMBER 8015814379	ELECTRONIC MAIL ADDRESS JDANDRAD@cc.utah.edu	FAX NUMBER 801 585-5361

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D. IDENTIFICATION AND SIGNIFICANCE OF THE OPPORTUNITY

There is rapidly growing public interest in aquaculture as a vehicle for enhancing food production, as sources of unique drugs and biochemical agents, as model systems for the study of toxins and toxin defense processes, as means to help treat wastes and deal with environmental problems, and to facilitate the development of closed and even remote life support systems (1-14).

Protein Solutions Inc. initiated work several years ago on the development of bioluminescent phytoplankton, single cell non-toxic dinoflagellates, for science education purposes. Our original rationale was that the beautiful and mysterious bioluminescence generated by such cultures would stimulate students, their teachers, and their parents to become interested in probing a wide range of scientific subjects. This has proven to be the case, and bioluminescent phytoplankton are now used by the Center for Integrated Science Education at the University of Utah for a wide range of workshops for inservice teachers and for the development of unique, integrated science courses on the University campus.

This activity has led to two product development efforts. Night Life™ is a science education kit for junior high and high school students utilizing *Pyrocystis lunula* and conventional tissue culture components. During the development of this product, we learned that this particular organism, and other dinoflagellates, can be maintained in semi-sealed gas permeable environments. This was a bit surprising because common wisdom suggests that these organisms are actually quite delicate and difficult to culture (17, 33, 34). In using these organisms to demonstrate the process of bioluminescence and protozoan biology to the general public, teachers, and students, we had to expose the organisms to less than ideal environments (22-24). In some cases they had to be shipped and kept in the dark for extended periods of time. In other cases they had to be completely sealed to eliminate possibility of spillage. In some cases they were exposed to adverse temperatures.

Based on the scientific literature and on our discussions with scientists knowledgeable in these organisms, we were very concerned that they would not survive such traumatic conditions. At the same time we were studying the phenomena of materially closed ecosystems--totally sealed systems in which life manages to co-exist in a microcosm or miniature ecosystem (27). We began to consider the possibility that perhaps the dinoflagellates could also develop their own micro ecosystem.

We now realize that most investigators greatly underestimated the durability and tolerability of certain of these organisms. We learned that cultures of certain dinoflagellates could exist for up to 24 months in a completely sealed environment. The cultures develop a balance between photosynthesis and respiration, which apparently allows them to reach a partial steady state and to survive in small volume sealed cultures for extended periods.

We also know that there are a variety of marine bacteria which co-culture with the organism of interest, thus there may be some symbiosis or at least balance among several organisms in the miniature ecosystem.

The fact that organisms that were considered frail, delicate, and difficult to culture are actually hardy, tolerant, and capable of survival in non-ideal situations and environments provides a unique set of opportunities.

bioluminescence (15-17, 21, 25, 33, 34), we selected two hardy, non toxic, bioluminescent dinoflagellates, which can be maintained in sealed environments for up to 6 months. All they require is eight to ten hours/day of light and moderate temperatures.

We originally selected dinoflagellates for our studies because they are the only marine protozoa which are bioluminescent. Although our interests were in the bioluminescence of dinoflagellates, as we have learned more and more about this unique class of organisms, we have become fascinated with their varied characteristics, their distribution throughout the globe, their unique properties and attributes, including bioluminescence, the production of toxins by some species, and their potential as a rich store of new and novel biochemical and possibly pharmaceutical agents (14-17).

There is a growing interest in dinoflagellates--and phytoplankton in general. The recent report describing a new and novel dinoflagellate responsible for East coast fish kills has stimulated much interest (40). The growing occurrences of red tides and algalblooms in many parts of the world is leading to greater and greater scientific and commercial interest in these organisms. The ability to culture and maintain various dinoflagellates in convenient, practical, safe, sealed containers, will be of growing interest to the research and commercial aquaculture community, as well as to the other constituencies noted above.

Protein Solutions, Inc. is committed to understanding and developing marine phytoplankton for a variety of purposes. Although our major, immediate product interests are in science education products, it is clear that the technology which the company is developing will be of interest to firms in the areas of aquaculture, biotechnology, and pharmaceuticals. We expect that this Phase I SBIR will demonstrate feasibility for larger volume cultures of a wider range of organisms and will lead to a Phase II effort, followed by contract research and development activities with those industries which need access to the unique science and technology which we are developing.

F. PHASE I TECHNICAL OBJECTIVES

1) Perform a thorough analysis and evaluation of the various types of transparent polymer films suitable to the culture of phytoplankton in sealed bags. Criteria for film evaluation and selection include: transport of O_2, CO_2, and water; transparency; mechanical strength; ease of sealing; surface properties of the film, particularly with respect to cell adhesion; and costs and availability.

2) The role of light intensity, light-dark cycle, and temperature on the culture of organisms in the sealed containers.

3) The composition of the culture medium. In order to optimize the longevity of the cultures, changes in the type and amount of nitrogen, phosphorous and the initial pH, will be evaluated.

4) Limited measurements of CO_2, O_2, H_2S, and CH_4 concentrations in the containers will be performed (in both gas and liquid phases) by Raman and IR spectroscopy.

5) An analysis of bag surface area, internal gas volume, and internal liquid volume will be performed, and the surface area and volume ratios will be optimized for various culture criteria: cell density, longevity, and bioluminescence intensity.

6) Given sufficient time and resources, the above studies will be extended to other phytoplankton, including diatoms.

G. PHASE I RESEARCH PLAN

Objective One: Polymer Materials:

Preliminary analysis by Mr. K. Foote of highly gas permeable polymer films for algae aquaculture applications (22) led to the selection of low density polyethylene for our initial studies (Table 1). Low density polyethylene has been used for the storage and propagation of plants in sealed environments (42).

Table 1: Best candidates for enclosing membrane material (from Ref. 22).*

Membrane Material	Gas Permeability mol*cm)/cm^2*sec*atm)*10-12® CO2	O2	Rate of Water Vapor Transport (g*mil)/(100in^2*24hr) at 37.88 C
Silicone rubber (Silastic)	290	140	170
Low density polyethylene	5.49	1.02	1.3
Ethylene-vinyl acetate copolymer	12.2	1.71	2.5
Fluorinated ethylene propylene copolymer (FEP TEFLON)	3.40	1.52	0.4

* Data from *Modern Plastics Encyclopedia*, Vol. 64, 1988, pp 553-557.

Basically one desires maximum CO_2 and O_2 permeability with minimum water vapor transport. The polymer with the highest gas permeability is silicone rubber, but that also has a very high rate of water vapor transport. Preliminary studies of cultures in silicone rubber bags showed a very rapid water loss, leading to increased salinity of the cultures to the point where cell death occurred. Silicone copolymers and silicones of varying cross-link density will be evaluated, however.

Low density polyethylene was selected because of the optimum combination of properties, because it is inexpensive, and because it is very easy to heat seal. Most of our experience to date has been with four mil thick polyethylene. We will examine a range of thicknesses and the trade off between thickness, mechanical properties, and gas transfer.

There are many types of low density polyethylene. We have not evaluated the various sources, various densities, crystallinities, and molecular weights. We also have not evaluated ethylene vinyl acetate copolymer, which is very promising and has a

We have now sealed small, low density cultures of the marine dinoflagellate *Pyrocystis lunula* in low density polyethylene bags. This material provides reasonable rates of oxygen and carbon dioxide transport while minimizing water loss from the closed culture. Although the culture appears to be "closed", it is actually exchanging O_2, CO_2 and water with its ambient environment, albeit the exchange is slower than in a normal culture vessel with an open air/water interface. The small volume cultures generally last for about six months, depending on lighting conditions, external temperature, and handling environments, although we have had some sealed bags viable for over 24 months! These small 30 to 40 ml volume cultures have only been superficially studied. We propose to study them in detail and develop a more complete understanding in this project. The small bag cultures are being used in a prototype science awareness/education product, Galaxsea™. This product is designed to introduce the phenomenon of bioluminescence, marine protozoa, and other topics to the general public.

We feel that there is a great potential for the development of larger volume, more optimized cultures enclosed in gas semi-permeable containers. That is the basis of this SBIR application. The availability of larger volume cultures of single-celled algae would be of significant interest for the following applications:

1) as food sources and/or supplements for marine aquaculture and marine aquaria (9, 10, 37);
2) as controlled and isolated cultures for basic biological research;
3) as controlled, isolated cultures for the biotechnological and pharmaceutical industry (11, 12);
4) as safe, isolated cultures for individuals studying these organisms for the production of toxins and other hazardous products; and
5) as means to prepare and deliver such organisms to other environments, including exhibition aquaria, home aquaria, research laboratories, and schools.

We propose to examine the various chemical and physical variables involved in optimizing the culture of marine microalgae in sealed, but gas permeable, environments.

E. BACKGROUND, RATIONALE, AND APPROACH

Protein Solutions, Inc., and its chief scientific officer, J.D. Andrade, have been working with bioluminescent microorganisms for about five years, the last two years involved in developing the Night Life™ and Galaxsea™ products previously noted. We have had extensive interaction with experts in the field, including those knowledgeable about dinoflagellates and other bioluminescent organisms. Our staff has attended the workshop on marine phytoplankton culture and techniques at the Provasoli Guillard Center for the Culture of Marine Phytoplankton in West Boothbay Harbor, Maine. Although R. Scheer, Principle Investigator, has limited marine biology experience, he has been working with these organisms for the past year and will be taking the Guillard culture course in autumn, 1994.

We have considerable experience with the culture of bioluminescent dinoflagellates in non-traditional media, including artificial sea waters (24), and have had experience on their culture in polyethylene bags (23). Dinoflagellates are normally considered to be delicate organisms which are difficult to culture and maintain (15-17). They also have a reputation of being toxic (15, 16). There are thousands of different dinoflagellates, some produce toxins, many others do not. Many are non-bioluminescent--others are. Many are fragile--some are hardy. After thorough consideration of the scientific literature and discussions with many experts on dinoflagellates and

different ratio of CO_2 to O_2 permeability, which would be very interesting in terms of fundamental studies of photosynthesis and respiration ratios.

Probably the best material is FEP Teflon. CO_2 and O_2 permeabilities are comparable to polyethylene, with water vapor transport three times less. Unfortunately FEP Teflon is extremely expensive for this application, although it has been used, and is marketed as a cell-culture bag for small volume research applications (41).

We propose to perform limited studies with FEP Teflon and silicone rubber, and much more extensive studies with various grades and types of low density polyethylene and ethylene vinyl acetate copolymer. We will also perform limited studies with polystyrene, polymethylmethacrylate, and polycarbonate. These polymers have extremely low gas permeability and water vapor transport rates, and can be considered materially closed containers if properly sealed. Polycarbonate is commonly used for phytoplankton culture (16, 17). These materials will serve essentially as controls for the approximately zero gas transfer and water vapor transfer rate studies.

We can readily monitor gas concentrations in the bag in both the gaseous and aqueous phases by Raman and infrared spectroscopies. J. Andrade's group at the University of Utah worked on Raman/gas analysis many years ago (43) and indeed these studies led to the development of a Raman/gas anesthesia analysis system which is now in commercial production by the Ohmeda Corporation. PSI has one of the Ohmeda "Rascal" Raman anesthesia monitoring systems and will be using that to measure the concentrations of the apolar gases, oxygen, nitrogen, and methane. The more polar gases, particularly CO_2, will be monitored by infrared absorption. The infrared equipment for these studies is available through PSI's affiliation with the University of Utah's Center for Biopolymers at Interfaces. This is described in more detail in the section on facilities and equipment.

Objective Two: Role of Light Intensity:

Our studies to date on light intensity have been qualitative, ranging from normal room lighting to more intense and controlled laboratory lighting--100 micro Einsteins/meter^2/sec. This is equivalent to two 40 watt fluorescent bulbs at about 1 ft. from the culture container. This is the typical intensity used in most culture laboratories working with photosynthetic microalgae (16, 17). Normal light cycles are 12 hours on and 12 hours off, which establishes typical circadian rhythm. Normal culture temperature is about 16° C.

Light intensities in the range of 10 to 200 micro Einsteins/m^2/second, temperatures in the range of 5 to 40° C., and the full range of light-dark cycles will be evaluated. Evaluation will consist of cell density in the bag, which will be measured by sampling and direct counting and indirectly measured by bioluminescence intensity and by chlorophyll absorption.

Objective Three: Media optimization:

Our preliminary studies indicate that a normal Guillard f/2 medium (17), but with the initial pH adjusted to about 7.5, will shift to an increased alkalinity after several months, plateauing at a pH of about 8.5 and maintaining that pH for several additional months. These results come from preliminary and quite qualitative studies. Modification of the initial pH of the medium and modification of the buffer type and capacity may have a significant effect on the pH changes during the life of the culture.

could even lead to the development of more complex ecosystems, possibly containing zoo-plankton, bioluminescent copepods, and other organisms. We are aware of the commercial novelty Ecosphere product, which is a completely enclosed culture of small organisms, including small shrimp.

Related R & D:

Protein Solutions, Inc. is committed to the science and application of bioluminescent dinoflagellates and other bioluminescent organisms for a variety of science education, biotechnological, and biomedical purposes. PSI has invested about $150,000 in research on bioluminescent dinoflagellates for science education.

In these preliminary studies, we have demonstrated that *Pyrocystis lunula* can be cultured and maintained in non laboratory environments, including homes, classrooms, and offices. We have found that no special lighting conditions or sources are required--normal room lighting and normal room temperatures are satisfactory. We have found that no special containers are required, only that they be transparent and clean (we routinely use glass apple juice containers and gallon polyethylene milk containers). We have found that the cultures can be maintained for up to 6 months in totally sealed containers, with no oxygen or CO_2 exchange (23, 24). We have found that the organisms exhibit no measurable acute toxicity and appear to be completely safe (28). We have found that their light/dark cycle/circadian rhythm can be very easily altered and programmed. We have found that they can be shipped by first class mail across the country with little difficulty, as long as they are delivered and opened within 5 days.

H. COMMERCIAL POTENTIAL

A very preliminary assessment of the potential applications, their markets and the distribution mechanisms suggests that phytoplankton bags are economically viable and have commercial potential. We have demonstrated their potential in the science education market. PSI's Galaxsea™ product and its Lunula Colony™ bags containing *Pyrocystis lunula* are being increasingly used for science education purposes. These living specimens allow a variety of hands-on experience and direct microscopic visualization without making direct contact with the culture, i.e., one can simply place the bags under a microscope and at low power directly visualize these large protozoa. These materials are now being distributed nationally to educators for science education purposes. These applications do not require considerable understanding of or control of the culture environment. The studies and applications proposed herein are designed to facilitate the more wide spread application of these cultures in more demanding applications.

The home hobby/marine aquarium market is significant. It is difficult to grow phytoplankton in conventional marine aquaria because of the gravel filtration systems commonly used (6). The availability of phytoplankton for filter feeders would facilitate the maintenance of a range of invertebrates and other organisms. Bags can be simply floated on the surface of the aquarium or maintained nearby, and a bag a day cut open and distributed to the aquarium for feeding purposes. One can also envision the use of larger bags and a simple controlled delivery mechanism for delivering the phytoplankton cultures to the aquarium for unattended feeding over a period of days to even weeks. This may be of particular interest in the display aquarium market.

We anticipate some interests in the pharmaceutical and biotechnological industries, largely in terms of the culture of toxin producing organisms for the study of

The typical media utilize nitrate and phosphate as the sources of nitrogen and phosphorous, respectively. Nitrate uptake is apparently balanced by OH production and increased alkalinity. This effect could be minimized by using ammonia as a nitrogen source, which leads to H+ generation, or perhaps even better by using the uncharged nitrogen compound, urea, in which pH changes are apparently minimal (18, 31, 19).

We will experiment with the media composition, including various ratios of nitrate, ammonia, and urea. pH will be routinely measured in both open and closed culture environments. We will also experiment with buffer changes in the media, particularly various ratios of the bicarbonate and phosphate buffer systems.

Objective Four: Measurement of O_2, CO_2, and pH (20, 21):

We have access to Raman and infrared spectroscopy equipment and thermal sensing modules and electrodes which enables us to perform measurements of pO_2 and pCO_2, as well as nitrate, ammonia, and phosphate in various culture environments. Gas phase CO_2 will be measured by standard IR absorption methods, whereas O_2 and N_2 in the gas phase will be measured by Raman Spectroscopy.

We have worked in the area of biosensors and in the development and application of biochemical sensors, using optical analysis techniques. It may be possible that some of these newer prototype biosensors may also be employed in this project. We regularly utilize Raman spectroscopy for gas measurement applications (43).

Objective Five: Area/Volume Effects:

Our present experience is based primarily on small, 3-inch by 4-inch polyethylene bags of *Pyrocystis lunula* in about 30 ml of Guillard f/2 medium with about 10 to 20 ml of enclosed gas volume. Such a container will maintain *Pyrocystis lunula* cultures for up to 6 months when maintained under ambient lighting and room temperature conditions. Viability has been assessed by the qualitative measurement of bioluminescence intensity, and in a few cases by direct cell observation in the optical microscope.

Surface to volume ratio analysis will be performed analytically, using the techniques developed in a preliminary fashion by Foote (22). In addition to surface-to-volume ratio studies using a typical bag geometry, we will perform preliminary experiments with more unique bag geometries, including elongated bags, cylindrical and spherical geometries.

Solution volumes will range from 10 to 5,000 ml, depending on the strength and mechanical characteristics of the bag materials and means of supporting the bags.

Objective Six: Other Phytoplankton:

Most of our experience to date has been with *Pyrocystis lunula*. We also maintain *Pyrocystis noctiluca* and *Pyrocystis fusiformis* in the laboratory, and will study these organisms as well. By discussions with dinoflagellate experts (15, 16), we will select a number of additional organisms, including several diatoms. The goal is to see just how versatile and general, and how widely applicable, CO_2 permeable containers are for the culture of dinoflagellates and other microalgae.

We will also do some preliminary work on mixed cultures, initially with *Pyrocystis lunula* and *Pyrocystis noctiluca*. It may well be that mixed culture studies

drugs and natural products derived from phytoplankton. In this way cultures can be maintained safely without fear of any direct human contact until they need to be sampled or otherwise used. The bags can be directly fitted with a silicone cap which would allow sampling with a syringe without the danger of direct contact. It is also possible that such bags could be used as detectors and sensors for toxic agents, although a means would have to be provided to deliver the toxic agents to the culture in the bag. This particular application is not addressed in this project, but is being seriously considered for other projects and other applications.

PSI is only now initiating a marketing study of the economic viability of these ideas and this technology. It is difficult to take this too far until we have a better understanding of the scientific basis and feasibility for the long term maintenance and viability of such sealed cultures, the objective of this SBIR application.

I. PRINCIPLE INVESTIGATOR/KEY PERSONNEL

Rob Scheer, Ph.D., Principle Investigator on this grant does not have a strong background in marine biology or aquaculture. Rob is a materials engineer with considerable background in the properties of plastics and materials, including some recent modeling studies on the gas permeability of polymeric materials. Dr. Scheer's biosketch is attached (next page).

He will be assisted by *Ms. Mara Lisonbee, a biology technician* with over two years experience in the culture of bioluminescent dinoflagellates. Both Mara and Rob will be taking the phytoplankton culture course offered by the Center for the Culture of Marine Phytoplankton and Dr. R. Guillard later this year.

Dr. Scheer's extensive background in polymer science and in general chemistry is directly relevant to Objectives 1, 2, and 5, whereas Ms. Lisonbee's background in phytoplankton culture will be focused primarily in Objectives 3 and 6.

In addition we have budgeted a full-time marine microbiologist post-doc for the project. It is expected that this individual to be appointed would have a strong background in biochemistry, as well as in marine biology.

Dr. Scheer will be assisted by *J.D. Andrade, Ph.D., President and Chief Scientific Officer* of Protein Solutions, who has been working on bioluminescence for the past 5 years and on bioluminescent dinoflagellates for the past 2 years. He has supervised PSI's work to date in the area of bioluminescent dinoflagellates. He has conducted courses for inservice elementary and high school teachers and has distributed *Pyrocystis lunula* cultures to at least 300 different teachers, friends, co-workers, and advisors for their input and critique. Joe has a strong interest in integrated science education. Dr. Andrade has been working on biomaterials and biotechnology problems for the past 25 years. He is an accomplished scientist with 5 books and over 100 peer-reviewed publication. Joe is also Director of the University of Utah's Center for Integrated Science Education (CISE). Although Joe is on the faculty of the University of Utah, he spends about 25% time with Protein Solutions, Inc., and will advise and assist on this project as needed. No funds are budgeted for his services. His vita is also attached.

A part-time graduate student and a part-time undergraduate are also budgeted to assist in the project.

Robert J. Scheer
390 Wakara Way, Room 31, Salt Lake City, UT 84112
Ph. (801)585-3128
e-mail (rob.scheer@m.cc.utah.edu)

EDUCATION

Ph.D. in Materials Science and Engineering, September 1993, University of Utah, Salt Lake City, UT. Dissertation emphasis: Mechanical, interfacial, and surface study of composite materials.

B.S. in Mechanical Engineering, 1989, Duke University, Durham, NC. GPA 3.76. Emphasis: Fracture mechanics and failure analysis of polymeric materials.

UNIVERSITY HONORS

National Science Foundation Fellow, University of Utah Graduate Research Fellow, Dean's List Duke University, Academic All American

Duke University Magna Cum Laude, Scholastic Societies: Tau Beta Pi and Pi Tau Sigma.

EXPERIENCE

Principle Investigator

Protein Solutions, Inc. Salt Lake City, UT. 1994 - present. Directed research for the design and implementation of novel science education materials.

Research Assistant

University of Utah, Salt Lake City, UT. 1989 - 1994. Tested mechanical properties of polymers and composites, studied surfaces and interfaces, tested adhesive bonds on the microscopic scale, and developed stress analyses related to materials testing.

Instructor/Tutor

University of Utah and Salt Lake Community College, Salt Lake City, UT. 1991 - present. Planned, instructed, and graded for undergraduate physical science classes. Served as tutor and teaching assistant.

Engineering Technician

Sandia National Laboratory, Albuquerque, NM. Summer, 1988 and Summer, 1989. Designed engineering experiments for failure analysis of ceramic materials, and extensively researched current experimental techniques for determining material fracture toughness. Designed engineering experiments for strength testing of brittle materials, and performed CAD. Interacted with diverse engineering disciplines on a major research project.

AFFILIATIONS: American Society for Mechanical Engineers, ASM International, The Minerals, Metals, and Materials Society, American Physical Society, The Center for Biopolymers at Interfaces

PUBLICATIONS:

Scheer, R.J. and J.A. Nairn. "Variational Mechanics Analysis of Stresses and Failure Analysis in Microdrop Debond Specimens." *Composites Engineering*, Vol. 2, No. 8, pp. 641-654, 1992.

Scheer, R.J. Ph.D. Dissertation, "An Energy Based Analysis of Fiber-Matrix Adhesion." University of Utah, 1993.

Andrade, J.D. and R.J. Scheer. "Applying 'Intelligent' Materials for Materials Education: The Lableess Lab™." *Proc., 2nd Annual Conference on Intelligent Materials*, Tech. Publ. Co., 1994, in press.

Advisors:

Dr. J.W. Hastings, Professor of Biology at Harvard University is one of the world's experts on dinoflagellate bioluminescence. Dr. Hastings serves on PSI's Scientific Advisory Board and will regularly provide input and guidance to the project.

Another member of PSI's Scientific Advisory Board is Mr. William Kelley. Mr. Kelley was one of the founders of Aquarium Systems, a corporation that sells artificial seawater (INSTANT OCEAN™) and related materials for hobbyists and exhibition aquaria. Mr. Kelley has also been involved in the design and development of exhibition aquaria nationally, including the Mystic Marine Life Aquarium in Mystic, Connecticut. He has retired in Salt Lake City and has been of immense help in the area of culture media for dinoflagellates.

Other dinoflagellate experts with whom Dr. Andrade has already consulted over the last 2 years and whom have offered their advice include Dr. Barbara Prezelin, Department of Biological Sciences, University of California, Santa Barbara; Dr. L. Brand, University of Miami, School of Oceanography, Miami; Dr. James Morin, Professor of Biology, , Los Angeles; Dr. Robert Guillard, the National Phytoplankton Culture Facility, West Boothbay Harbor, Maine.

J. CONSULTANTS

None.

K. FACILITIES AND EQUIPMENT

The work on this project will be carried out by PSI, Inc., in its laboratories located at 350 West 800 North, Suite 218, Salt Lake City, Utah 84103. PSI is a member of the Center for Biopolymers at Interfaces at the University of Utah, one of the State's Centers of Excellence (see p 17). PSI is also a key corporate participant in the University's Center for Integrated Science Education (CISE). PSI has a Technology Transfer agreement with the University of Utah Research Foundation. PSI's laboratories are equipped to perform the necessary biological, chemical, engineering, and evaluation studies. The laboratory space (1100 ft^2) includes a culture room and a general biology lab. Normal office and instrument room space is also present. Sophisticated equipment which may be required may be used by our team at the University of Utah (see next page).

L. CURRENT AND PENDING SUPPORT

Current Support:

NSF Phase I SBIR, The Lableless Lab™ in Polymer Materials (ends 8/15/94) Phase II will be submitted by 9/15/94.

Pending Support:

1. Department of Energy SBIR, Luminescent Films based on Photoproteins (7/1/94 - 1/1/95) two man months.

2. NSF STTR, Direct Reading, Quantitative Biosensors for ATP-Dependent Processes &7/1/94 - 6/30/95) two man months.

3) NIH SBIR, Direct Reading, Quantitative Bioluminescent Biosensors, (10/1/94 - 4/1/95) on man month.

As PSI's sponsored R & D volume increases several Ph.D. level research personnel will be attracted and hired, although Dr. Scheer will serve as principle investigator and primary supervisor and scientist for these projects, the more routine scientific and engineering activities and tasks will be delegated to these new staff researchers. Dr. Scheer will retain a minimum of one man month of direct involvement with each of the projects for which he is serving as principle investigator.

PSI has invested $150,000 (provided primarily by its founders and major stock holders) in the initial studies and product development. PSI expects to continue funding the project from stock-derived funds. PSI is now discussing equity investments by a number of local investors and investment groups.

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M. EQUIVALENT PROPOSALS TO OTHER FEDERAL AGENCIES:

None.

N. BUDGET AND JUSTIFICATION

The budgeting and responsibility of the various personnel were discussed earlier in the Personnel section. Dr. Scheer will spend one person month on this project.

Travel funds are requested for one of our staff to attend the Phytoplankton/Toxic Algal Blooms Conference. Funds are also requested for Dr. Scheer and Ms. Lisonbee to attend the Guillard Culture Course later this year in Maine.

Instrument time/analytical services funds refers to the use of equipment on a fee for service basis at the University of Utah via our affiliation with the Center for Biopolymers at Interfaces discussed earlier.

PSI requests indirect costs of 35% of total direct costs.

O. PRIOR PHASE II AWARDS

None.

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