Tech Transfer (17)

U-1826
(Leave Blank)

INVENTION DISCLOSURE

Date of Disclosure 7-5-92 Date Invention Complete _____

Inventor(s) and Department(s):

J. D. Andrade - Bioengineering
John D. Tolber, Jr. - Bioengineering

Main Contact's Name Joe Andrade, and Phone Number 1-4379

Invention Title:

Culture of Marine Phytoplankton in Sealed Environments

Brief Summary of Invention (paper, fuller descriptions, etc., may be appended, but please also give a brief summary):

We claim the culture of marine phytoplankton in completely sealed environments. We claim the culture of marine phytoplankton in containers which permit the exchange and transport of O_2, CO_2, and water vapor. We claim the culture of bioluminescent dinoflagellates in these environments and conditions. We claim the culture of phytoplankton in sealed containers of high optical clarity as living specimens for microscopy, science education, and related applications.

Advantages over State-of-the-Art:

Phytoplankton have been cultured in the laboratory for decades. They are often considered somewhat delicate or difficult to culture, but a variety of techniques, methods, and media have been developed, which allow fairly routine culture of these organisms. Common wisdom is that these organisms require exposure to atmospheric carbon dioxide and a saline water environment, with appropriate metal and vitamin nutrients. 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. We now realize that those skilled in the art had greatly underestimated the durability and tolerability of certain of these organisms.

Practical Applications:

The ability of phytoplankton to grow in sealed environments opens a wide range of possible applications, including the development of living specimens for science education, for environmental studies, and as detectors and sensors for various agents. In addition these cultures are useful as novelty products, particularly in the case of bioluminescent organisms. They are also of interest as products and exhibits in marine aquaria and science centers. They are also applicable as living foods for the hobbyist aquarium and even the aquaculture business.

Dates(s) of Conception: _____, and Proof-of-Principle of Invention: _____

Are there written records of these dates? ✓ If yes, where? Protein Solutions, ANC IPSI Notes; Shareholders Meeting Minutes

Have the essential elements of the invention been disclosed to anyone outside the University, either orally or in writing? Yes. If yes, please give date and details: May, 1992 Utah academy of Arts & Sciences Meeting (see attached abstract)

Do you intend to disclose the essential elements of the invention in the future, either orally or in writing? No If yes, please give details and probable date of future disclosure (e.g., publication):

Please list below all sources of funding for materials, equipment and/or manpower involved in making the invention (check where appropriate):

1.___ All funds from unrestricted University/Departmental budget.

2. ✓ Some /all (circle one) funds from federal or non-profit granting agencies:

Agency _____ Grant No. _____ Univ. Acct. No._____

Agency _____ Grant No. _____ Univ. Acct. No._____

Agency _____ Grant No. _____ Univ. Acct. No._____

3. ✓ Some /all (circle one) funds from other companies or organizations.

Company/Organization: Protein Solutions, Inc

Grant or Contract No. Acct S-21004

Please list any commercial companies you feel are/should be interested in your discovery (names of individuals at companies are extremely helpful): Protein Solutions, Inc

The undersigned hereby declare(s) that they (he/she) are (is) the true and only originator(s) of the invention disclosed herein, and that the invention arose in the course of work at or on behalf of the University of Utah (all inventors should sign).

Inventor 1 _____ Inventor 2 _____

Inventor 3 _____ Inventor 4 _____

Inventor 5 _____ Inventor 6 _____

TECH. INTEREST CATEGORIES: (LEAVE BLANK) _____ _____ _____ _____ _____

_____ _____ _____ _____ _____

THE UNIVERSITY OF UTAH

August 18, 1992

TO: J. D. Andrade, Bioengineering - 2480 MEB

FROM: Tom Major, Director

RE: CULTURE OF MARINE PHYTOPLANKTON IN SEALED ENVIRONMENTS [Inventors: J. D. Andrade and John D. Tobler, Jr.] U-1826

ENCL.

CC: John D. Tobler, Jr.

Dear Joe:

Referenced above is the new case number for your recent disclosure. A set of 'green sheets' is enclosed for your completion and signature, by both you and your co-inventor. We need to address some patent issues here, please give me a call when you get a chance. Thanks.

Tom

Technology Transfer Office
421 Wakara Way, Suite 170
Salt Lake City, Utah 84108
(801) 581-7792
FAX: (801) 581-7539

~~Patent~~
Tech Transfer
(17)

Invention Disclosure

Culture of Phytoplankton in Sealed Environments

Center for Integrated Science Education and
Department of Bioengineering
University of Utah

By

J.D. Andrade and J. Tobler

Phytoplankton are small photosynthetic organisms that generally live on the surface of the world's oceans, seas, and lakes.

Marine phytoplankton require saline environments and are common on the surface of all the world's oceans and marine seas. They are often considered the primary food in the seas and indeed in the overall food chain. They generally multiply by cell division and depend on photosynthesis as their main energy source.

Phytoplankton have been cultured in the laboratory for decades. They are often considered somewhat delicate or difficult to culture, but a variety of techniques, methods, and media have been developed, which allow fairly routine culture of these organisms (1,2). Indeed there is a national facility for the preservation and culture of marine phytoplankton in West Boothbay Harbor, Maine(3). This facility stocks and maintains standard cultures of various marine phytoplankton for distribution to scientists and researchers.

Common wisdom is that these organisms require exposure to atmospheric carbon dioxide and a saline water environment, with appropriate metal and vitamin nutrients. Phytoplankton have been used in aquaculture installations as food for crustaceans, copepods, and zooplankton. They are even used in various public aquaria for such purposes (4,5).

They are grown and studied by scientists, and they are of interest as an indicator of productivity in the oceans and possibly even as an indicator of global warming and the greenhouse effect (6).

Many phytoplankton have various survival mechanisms to maintain their existence even under adverse environmental conditions. The most common such mechanism is the formation of cysts. Cysts survive on the ocean bottom until the conditions become satisfactory again, at which time excystment occurs and the phytoplankton population redevelops (7,8).

We have been studying several marine phytoplankton (dinoflagellates) which are bioluminescent. They produce light at night when mechanically stimulated. Our studies on these organisms initially employed the conditions which research scientists and experts had defined as optimum for culture and growth (1,2,7).

In using cultures of these organisms to demonstrate the process of bioluminescence to the general public, to teachers, and students, we had to expose the organisms to less than ideal environments (9). 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 any possibility of spillage. In some cases they had to be 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 (10). We began to consider the possibility that perhaps the dinoflagellates could also develop their own micro ecosystem.

We now realize that those skilled in the art had greatly underestimated the durability and tolerability of certain of these organisms.

We discovered that cultures of certain dinoflagellates could exist for 6 months or more 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 6 months or more.

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. Nevertheless, the discovery is that organisms that were considered frail, delicate, and difficult to culture have been shown to be hardy, tolerant, and capable of survival in situations and environments which were not predicted.

Based on this discovery, we have performed another series of studies in which these organisms have been sealed in polyethylene bags. The rationale here was that the polyethylene at least allows some limited transport of oxygen and CO_2, through the bag. The organisms would therefore be at least as tolerant of this environment as of the completely sealed flask environment in which the original experiments were performed. This has proven to be the case. Organisms sealed in simple polyethylene bags actually multiply and poliferate for some period of time, and then appear to maintain themselves in a steady state for several months. The life of these cultures is of the order of 3-6 months, roughly comparable to that in the sealed flasks (11).

The ability of phytoplankton to grow in sealed environments opens a wide range of possible applications, including the development of living specimens for science education, for environmental studies, and as detectors and sensors for various agents.

The only conditions the cultures require is moderate temperatures, moderate light levels for 6-10 hours a day, and moderate handling; mechanical activation or stimulation should not be excessive. 6-14 hrs/day.

In addition these cultures are useful as novelty products, particularly in the case of bioluminescent organisms. They are also of interest as products and exhibits in marine aquaria and science centers. They are also applicable as living foods for the hobbyist aquarium and even the aquaculture business.

Important variables affecting the longevity of a sealed phytoplankton culture include initial cell density, air volume to liquid volume ratio in the container, initial CO_2 level and pH, transparency of the container, initial composition of the seawater medium, mechanical stimulation and disturbances during the course of the culture, temperature, light intensity and light duration, details of the day/night cycle, the specific

phytoplankton involved, bacteria and other organisms present in the culture, the nature of the material housing the culture (the flask or the bag), gas transport characteristics of that material water transport characteristics of that material, the particular chemical composition of the material from which the container is made, especially with respect to the ability of the organisms to attach or not attach to the walls of the container, and whether or not the container releases or binds chemicals to or from the culture medium, respectively.

We claim the culture of marine phytoplankton in completely sealed environments. We claim the culture of marine phytoplankton in containers which permit the exchange and transport of O_2, CO_2, and water vapor. We claim the culture of bioluminescent dinoflagellates in these environments and conditions. We claim the culture of phytoplankton in sealed containers of high optical clarity as living specimens for microscopy, science education, and related applications.

References:

1. A. Sournia, ed., Phytoplankton Manual, UNESCO, 1978
2. R.R.L. Guillard, "Culture of Phytoplankton..." in C.J. Berg, Jr., *Culture of Marine Invertebrates*, Hutchinson Ross Publ. Co., 1983; see also his Chapter in Ref. 9.
3. Center for the Culture of Marine Phytoplankton, West Boothbay Harbor, Maine.
4. S. Spotte, Captive Seawater Fishes, Wiley, 1992.
5. W.H. Adey and K. Loveland, Dynamic Aquaria, Academic Press, 1991.
6. B. Prezelin in Ref. 8.
7. D.L. Spector, ed. Dinoflagellates, Academic Press, 1984.
8. F.J.R. Taylor, ed. Biology of Dinoflagellates, Blackwell, 1987.
9. John Tobler and J. Andrade, "Culture of Bioluminescent Dinoflagellates in Non-Traditional Media", Abstract, Utah Academy of Arts and Sciences, Salt Lake City, May 1991. (attached)
10. C.E. Folsome and J.A. Hanson, "Emergence of Material-Closed-System Ecology", in N. Polunin, ed., Ecosystem Theory and Application, Wiley, 1986, pp. 269-288.
11. J. Tobler and J.D. Andrade, "Culture of Pyrocystis Lunula in Sealed Polyethylene Bags", Abstract, Utah Academy of Arts and Sciences, May, 1992. (attached)

J.D. Andrade
Professor

7/1/92
Signature
Date

J. Tobler
Laboratories Assistant

May 11, 1992 (dictated)

Encl: Two Abstracts
Ref. 9 and 11.

T061-1

Abstract

Utah Academy of Sciences, Arts, and Letters
Spring Meeting, May 10, 1991
Westminster College, Salt Lake City, Utah

Culture of Bioluminescent Dinoflagellates in Non-Traditional Media

by J. Tobler and J. Andrade, Department of Bioengineering
University of Utah, Salt Lake City, Utah 84112

Certain marine microalgae, dinoflagellates, give off light when mechanically stimulated. This natural bioluminescence is responsible for the light seen on the surfaces of oceans, bays, and estuaries throughout the tropical and sub-tropical regions of the world, and often in temperate climates as well. Pyrocystis lunula and Pyrocystis noctiluca are two such dinoflagellates which have high bioluminescence intensities and are relatively easy to grow and maintain in laboratory environments. When mechanically stimulated, they produce a bright bioluminescent flash. We have been evaluating these organisms as possible science education aids for discovery oriented science curricula.

Historically the organisms have been grown in natural seawater, supplemented with the Guillard F/2 medium, a medium which was designed for the culture of marine phytoplankton. Because Utah is far from natural ocean waters, and because natural sea waters vary dramatically at various times of the year in various locations, we are evaluating various synthetic, artificial sea waters, and Great Salt Lake waters for dinoflagellate culture applications.

Progress on the work to date will be reviewed, including a listing of the artificial sea salts employed, dinoflagellate growth rates, bioluminescence intensities, and culture stability and longevity.

Acknowledgements:

This work has been supported by Protein Solutions, Inc., Salt Lake City.

AG-Tobl-2
U. 1992

Abstract
for submission to
Utah Academy of Arts and Sciences
Annual Meeting
May 1992

Culture of Pyrocystis Lunula in Sealed Polyethylene Bags

J. Tobler and J.D. Andrade
Center for Integrated Science Education (CISE)
Department of Bioengineering
2480 MEB
University of Utah
Salt Lake City, Utah 84112

Pyrocystis lunula is a bioluminescent, non toxic dinoflagellate which is beginning to be utilized for science education purposes. NIGHT-LIFE™, a commercial science education kit, utilizes pyrocystis lunula and conventional tissue culture flasks using a modified Guillard F/2 culture medium. Although this approach works very well, it is also desirable to maintain the cultures under totally sealed conditions. A totally sealed culture permits students to observe the development of the culture without having to take samples, pour solutions, or otherwise disturb the colony. As these organisms are photosynthetic, and require CO2 and O2 exchange to live, it has always been doubtful whether they could exist in a sealed environment.

Experience with the NIGHT-LIFE™ product demonstrated a 3 month shelf life with pyrocystis lunula flasks which were completely sealed with no gas exchange with the surrounding environment. Experience with the gas transfer characteristics of membranes led to a study where the colony was completely sealed in thin polyethylene bags. The colonies have survived in this sealed culture environment for several months with little problem.

There is an increase in salinity due to progressive water loss by water vapor permeation through the bags. The bags always feel cool, presumably due to evaporative cooling. Though there are other polymers, such as polydimethyl siloxane, with better gas transfer characteristics, they also have greater water loss.

The bags have the advantage that the organisms in the colony can be observed readily. They can be placed directly in an optical microscope. A variety of temperature, light intensity, light period, and related experiments can readily be performed without any direct contact with the culture or the organisms. Completely sealed pyrocystis lunula colonies in polyethylene bags are now the basis of a new product development, LIGHT-BAG™.

This work was partially supported by Protein Solutions, Inc., Salt Lake City, Utah. NIGHT-LIFE™, LIGHT-BAG™, LIGHT-POUCH™, FLIGHT BAG™, are trademarks of Protein Solutions, Inc.

Protein Solutions, Inc. (PSI)
Science Education Innovators

Dear Bill

MEMO

To: William S. Britt
Tom Major

From: J.D. Andrade

Date: 2 April, 1993

Subject: Patent Application Draft

Enclosed is a draft of the patent application, "Culture, Maintenance, Storage, and Transport of Phytoplankton in Sealed Environments."

This is a disclosure that was made nearly 1 year go, and for which we must submit a patent application with in the next month.

I apologize for the delay in getting this draft to you, but I think it is relatively complete and that we can proceed with what should be a simple and short application.

I enclose a hard copy; a Macintosh disk containing this draft is enclosed for Mr. Britt.

Please let me know what else I can do to facilitate this submission.

psi/apr1

390 Wakara Way, Room 31
University of Utah Research Park
Salt Lake City, Utah 84108
(801) 585-3128

Patent Application

Title: Culture, Maintenance, Storage, and Transport of Phytoplankton in Sealed Environments

Inventors: Joseph D. Andrade and John Tobler

Assignee: The University of Utah, Salt Lake City, UT 84112

Application Number:

Filed:

References Cited:

1. A. Sournia, ed., Phytoplankton Manual, UNESCO, 1978.
2. S. Spotte, Captive Seawater Fishes, Wiley, 1992.
3. D. L. Spector, ed., Dinoflagellates, Academic Press, 1984.

Abstract:

A method is disclosed for the culture of marine phytoplankton using transparent or semi transparent, gas permeable containers and natural or artificial sea water media. Such cultures are maintained indefinitely if given appropriate light and temperature conditions.

Sealed in polyethylene or other plastic bags, the cultures are very easy to handle, can be directly observed without direct contact, and are applicable to a wide variety of scientific, technical, education, and entertainment purposes.

Background:

Phytoplankton are small photosynthetic organisms that generally live on the surface of the world's oceans, seas, and lakes.

Marine phytoplankton require saline environments and are common on the surface of all the world's oceans and marine seas. They are often considered the primary food in the seas and indeed in the overall food chain. ~~They generally~~ multiply by cell division and depend on photosynthesis as their main energy source. — small cell phytoplankton often

Phytoplankton have been cultured in the laboratory for decades. They are often considered somewhat delicate or difficult to culture, but a variety of techniques, methods, and media have been developed which allow fairly routine culture of these organisms. There is a national facility for the preservation and culture of marine phytoplankton which stocks and maintains standard cultures of various marine phytoplankton for distribution to scientists and researchers. Common wisdom is that these organisms require exposure to atmospheric carbon dioxide and a saline water environment, with appropriate metal and vitamin nutrients.

They are grown and studied by scientists. They are of interest as an indicator of productivity in the oceans and possibly even as an indicator of global warming and the greenhouse effect.

Phytoplankton are used in aquaculture installations and public aquaria as food for crustaceans, copepods, and zooplankton.

We have studied marine phytoplankton (dinoflagellates) which are bioluminescent. They produce light at night when mechanically stimulated. Our studies on these organisms initially employed the conditions which research scientists and experts had defined as optimum for culture and growth.

In using cultures of these organisms to demonstrate the process of bioluminescence to the general public, to teachers, and students, we had to expose the organisms to less than ideal environments. 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 any possibility of spillage. In some cases they had to be 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.

Based on our knowledge of the gas transfer properties of synthetic polymer films, we hypothesize that phytoplankton might tolerate containment in sealed polymer bags, permitting oxygen and carbon dioxide transport and at least partial transparency to light.

The scientific literature on the culture of these organisms generally cautions that they are delicate and require optimum laboratory conditions in order to successfully grow. Those skilled in the art advise that such organisms would not grow in plastic bag environments.

We have therefore discovered and applied a novel, highly practical, and convenient means to culture phytoplankton in sealed environments.

Example:

A six inch length of standard 2 mil thick, 2 in wide, low density polyethylene tubing is cut to a six inch length and heat sealed at one end using a common film heat sealer. The bag is then held vertically and approximately 30 cc's of marine phytoplankton suspension is dispensed into the bag. The top of the bag is then heat sealed such that the internal contents contains about 30cc of the aqueous cell suspension and an additional 30 cc of air. Such a bag is completely sealed and ~~dried and is now~~ treated as a typical flask culture is treated, ~~that is it is~~ given light under normal culture illumination conditions for 6-14 hours per day. The phytoplankton cells which have been studied include pyrocystis lunula, pyrocystis noctiluca, and pyrocystis fusiformis. The same procedure can be used for bags of very different sizes ranging from one centimeter tubes to 12 in tubes and ranging in length from 2 cm to 2 ft, together with comparable volumes.

Polyethylene thicknesses from ~~1 to 5~~ 1 to 10 mil are appropriate, although 2-4 mil is preferred.

Discussion:

We discovered that cultures of certain dinoflagellates could exist for over 12 months or more in a completely sealed environment. The cultures develop a balance between photosynthesis and respiration, which allows them to reach a partial steady state and to survive in small volume sealed cultures for over a year.

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. Nevertheless, the discovery is that organisms that were considered frail, delicate, and difficult to culture have been shown to be hardy, tolerant, and capable of survival in situations and environments which were considered intolerable by those skilled in the art.

The ability of phytoplankton to grow in sealed environments opens a wide range of possible applications, including the development of living specimens for science education, for environmental studies, and as detectors and sensors for various agents.

The cultures require moderate temperatures, moderate light levels for 6-14 hours a day, and moderate handling; mechanical activation or stimulation should not be excessive.

These cultures are useful as novelty products, particularly in the case of bioluminescent organisms. They are also of interest as products and exhibits in marine aquaria ~~and~~ science centers. They are also applicable as living foods for the hobbyist aquarium and the aquaculture business.

Important variables affecting the longevity of a sealed phytoplankton culture include initial cell density, air volume to liquid volume ratio in the container, initial CO_2 level and pH, transparency of the container, initial composition of the seawater medium, mechanical stimulation and disturbances during the course of the culture, temperature, light intensity and light duration, details of the day/night cycle, the specific phytoplankton involved, bacteria and other organisms present in the culture, the nature of the material housing the culture (the flask or the bag), gas transport characteristics of that material, water transport characteristics of that material, the particular chemical composition of the material from which the container is made, especially with respect to the ability of the organisms to attach or not attach to the walls of the container, and whether or not the container releases or binds chemicals to or from the culture medium.

Claims:

We claim

1: The isolation and sealing of ~~marine~~ phytoplankton in transparent or semi-transparent containers which permit the permeation of oxygen and carbon dioxide. Such containers ranging in size from 0.1 milliliters to 100 liters and ranging in transparency from perfect optical transparency to weakly translucent.

2: We claim the sealing and growth of ~~marine~~ phytoplankton in low-density polyethylene ranging from 0.5 to 10 mil in thickness, and in sizes ranging from 0.1 milliliter to 1 liter, said polyethylene sealed by conventional techniques.

3: We claim the culture of phytoplankton in sealed containers consisting of other gas permeable materials, including silicone rubber, ethylene-vinylacetate copolymers, fluorinated ethylene propylene copolymer, other fluorinated and partially fluorinated polymers, and micro porous polymers.

Law Offices
TRASK, BRITT & ROSSA
(a professional corporation)

David V. Trask
William S. Britt
Thomas J. Rossa
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December 28, 1993

SENT BY CERTIFIED MAIL

Mr. Tom Major, Director
University of Utah
Technology Transfer Office
421 Wakara Way
Suite 170
Salt Lake City, UT 84108

Re: U.S. PATENT APPLICATION

Invention	CULTURE, MAINTENANCE, STORAGE, AND TRANSPORT OF PHYTOPLANKTON IN SEALED ENVIRONMENTS
Inventors	Joseph D. Andrade and John D. Tobler, Jr.
Applicant/Assignee	University of Utah
Serial No.	08/056,168
Filing Date	April 30, 1993
Your Reference No.	U-1826
Our Case No.	2249

Dear Tom:

Enclosed is the original University of Utah Research Foundation Assignment which has been recorded and returned by the Patent and Trademark Office in connection with the above-referenced application. You will note that the Assignment was recorded on November 1, 1993, reel 6746, frames 310 through 313. Also enclosed is a copy of the Official Notice of Recordation.

The assignment is like a deed and should be stored in a secure location.

Very truly yours,

Allen C. Turner

ACT/le
Enclosure: Original Assignment
Official Notice of Recordation

C:\LETTERS\4Q93\2249.URF(LE_) 12/28/93

UNITED STATES DEPARTMENT OF COMMERCE
Patent and Trademark Office
ASSISTANT SECRETARY AND COMMISSIONER
OF PATENTS AND TRADEMARKS
Washington, D.C. 20231

DATE: 11/30/93
TO:

N09A

ALLEN C. TURNER
TRASK, BRITT & ROSSA
P.O. BOX 2550
SALT LAKE CITY, UT 84110

UNITED STATES PATENT AND TRADEMARK OFFICE
NOTICE OF RECORDATION OF ASSIGNMENT DOCUMENT

THE ENCLOSED DOCUMENT HAS BEEN RECORDED BY THE ASSIGNMENT BRANCH OF THE U.S. PATENT AND TRADEMARK OFFICE. A COMPLETE MICROFILM COPY IS AVAILABLE AT THE U.S. PATENT AND TRADEMARK OFFICE ON THE REEL AND FRAME NUMBER REFERENCED BELOW.

PLEASE REVIEW ALL INFORMATION CONTAINED ON THIS NOTICE. THE INFORMATION CONTAINED ON THIS RECORDATION NOTICE REFLECTS THE DATA PRESENT IN THE PATENT ASSIGNMENT PROCESSING SYSTEM. IF YOU SHOULD FIND ANY ERRORS OR QUESTIONS CONCERNING THIS NOTICE, YOU MAY CONTACT THE EMPLOYEE WHOSE NAME APPEARS ON THIS NOTICE AT 703-308-9723. PLEASE SEND REQUEST FOR CORRECTION TO: U.S. PATENT AND TRADEMARK OFFICE, ASSIGNMENT BRANCH, NORTH TOWER BUILDING, SUITE 10C35, WASHINGTON, D.C. 20231

ASSIGNOR: DOC DATE: 10/14/93
UNIVERSITY OF UTAH

RECORDATION DATE: 11/01/93 NUMBER OF PAGES 004 REEL/FRAME 6746/0310

DIGEST : ASSIGNMENT OF ASSIGNORS INTEREST

ASSIGNEE:
UNIVERSITY OF UTAH RESEARCH FOUNDATION
210 PARK BUILDING
SALT LAKE CITY, UT 84112

SERIAL NUMBER 8-056168 FILING DATE 04/30/93
PATENT NUMBER ISSUE DATE 00/00/00

EXAMINER/PARALEGAL
ASSIGNMENT BRANCH
ASSIGNMENT/CERTIFICATION SERVICES DIVISION

DEC 2 2

ASSIGNMENT

In consideration of One Dollar ($1.00), and other good and valuable consideration, the receipt of which is hereby acknowledged, the University of Utah ("ASSIGNOR"), hereby:

Sells, assigns and transfers to the University of Utah Research Foundation, ("ASSIGNEE"), a non-profit organization existing under the laws of the State of Utah, its successors, assigns and legal representatives, the entire right, title and interest for the United States and all foreign countries, including priority rights, in and to any and all improvements for which an application for United States Letters Patent entitled

"CULTURE, MAINTENANCE, STORAGE, AND TRANSPORT OF PHYTOPLANKTON IN SEALED ENVIRONMENTS"

has been filed under Serial No. 08/056,168, on April 30, 1993, and in and to said application and all divisional, continuing, substitute, renewal, reissue, and all other applications for Letters Patent which have been or shall be filed in the United States and all foreign countries on any of said improvements; and in and to all original and reissued patents which have been or shall be issued in the United States and all foreign countries on said improvements;

Agrees that said ASSIGNEE may apply for and receive Letters Patent for said improvements in its own name; and that, when requested, without charge to but at the expense of said

ASSIGNEE, its successors, assigns and legal representatives, to carry out in good faith the intent and purpose of this assignment, the undersigned will execute all divisional, continuing, substitute, renewal, reissue, and all other patent applications on any and all said improvements; execute all rightful oaths, assignments, powers of attorney and other papers; communicate to said ASSIGNEE, its successors, assigns, and representatives, all facts known to the undersigned relating to said improvements and the history thereof; and generally do everything possible which said ASSIGNEE, its successors, assigns or representatives shall consider desirable for aiding in securing and maintaining proper patent protection for said improvements and for vesting title to said improvements and all applications for patents and all patents on said improvements, in said ASSIGNEE, its successors, assigns and legal representatives; and

Covenants with said ASSIGNEE, its successors, assigns and legal representatives that no assignment, grant, mortgage, license or other agreement affecting the rights and property herein conveyed has been made to others by the undersigned, and

that full right to convey the same as herein expressed is possessed by the undersigned.

ASSIGNOR:

UNIVERSITY OF UTAH

(Signature) _____ Date: October 14, 1993

Name: Tom Major

Title: Director, Tech. Trans. Ofc.

STATE OF UTAH)
: ss.
County of Salt Lake)

Before me this 14th day of October 1993, personally appeared Tom Major, the person who is described in and who executed the above instrument, and acknowledged to me that he executed the same of his own free will for the purpose therein set forth.

_____
Notary Public
Residing at: Salt Lake City, UT

My Commission Expires:

April 8, 1997

NOTARY PUBLIC
Peggy N. Halliday
421 Wakara Way #170
Salt Lake City, Utah 84108
My Commission Expires
April 8, 1997
STATE OF UTAH

N:\0274\2249.ASN(LE_) 10/12/93

RECEIVED FEB 13

PATENT & TRADEMARK

NOV - 1 1993

Law Offices
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(a professional corporation)

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Laurence B. Bond
Joseph A. Walkowski
E. Russell Tarleton
Allen C. Turner
Alan K. Aldous†
Julie K. Morriss
A. John Pate
registered patent attorneys
†admitted in California, Oregon and Washington

Susan E. Sweigert, Ph.D.
registered patent agent

October 13, 1993

Mr. Tom Major, Director
Technology Transfer Office
421 Wakara Way
Suite 170
Salt Lake City, Utah 84108

Re: U.S. PATENT APPLICATION

Invention	CULTURE, MAINTENANCE, STORAGE, AND TRANSPORT OF PHYTOPLANKTON IN SEALED ENVIRONMENTS
Inventor	Andrade & Tobler
Applicant/Assignee	University of Utah
Serial No.	08/056,168
Filing Date	April 30, 1993
Your Docket No.	U-1826
Our Case No.	2249

Dear Tom:

Enclosed is an as-filed copy of the above-identified U.S. patent application. Also enclosed is a copy of the official filing receipt which contains the filing date and serial number assigned. This information should be kept confidential so that others cannot use it to your detriment. You may now properly mark the invention either "Patent Pending" or "Patent Applied For."

To preserve the benefit of the U.S. filing date, corresponding foreign applications (in convention member countries) must be filed within 1 year of the U.S. filing date, i.e., before April 30, 1994.

Also enclosed is an assignment from the University of Utah to the University of Utah Research Foundation. Please execute the assignment before a notary and return it to us for filing. We will forward it to the Patent office for recordation as soon as it is received.

As soon as we receive anything further in connection with this application, we will let you know.

Very truly yours,

Allen C. Turner

ACT/le

Enclosures: As-filed copy of patent application
Copy of official filing receipt
Assignment

cc: William S. Britt

C:\LETTERS\4093\2249.LTR(LE_) 10/13/93

PATENT
Attorney Docket 2249

APPLICATION FOR LETTERS PATENT

for

CULTURE, MAINTENANCE, STORAGE, AND TRANSPORT OF PHYTOPLANKTON IN SEALED ENVIRONMENTS

Inventor:
Joseph D. Andrade
John Tobler

Attorney:
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Registration No. 20,969
TRASK, BRITT & ROSSA
P.O. Box 2550
Salt Lake City, Utah 84110
(801) 532-1922

CERTIFICATE OF MAILING

I hereby certify that this paper or fee along with any attachments referred to or identified as being attached or enclosed is being deposited with the United States Postal Service

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on the date of deposit shown below with sufficient postage and in an envelope addressed to the Commissioner of Patents and Trademarks, Washington, D.C. 20231.

4/30/93
Date of Deposit

Signature of registered practitioner or other person having reasonable basis to expect mailing to occur on date of deposit shown pursuant to 37 C.F.R. §1.8(a) or person actually making deposit pursuant to 37 C.F.R. §1.10(b)

Jedd Garrett
Typed/printed name of person whose signature is contained above

CULTURE, MAINTENANCE, STORAGE, AND TRANSPORT OF PHYTOPLANKTON IN SEALED ENVIRONMENTS

BACKGROUND OF THE INVENTION

Field of the Invention: The invention relates to self-contained, storage-stable cultures of photosynthetic microorganisms and, in particular, to containment means for storage and shipping of such microorganisms.

State of the Art: Phytoplankton are small photosynthetic organisms that generally live on the surface of the world's oceans, seas, and lakes.

Marine phytoplankton require saline environments and are common on the surface of all the world's oceans and marine seas. They are often considered the primary food in the seas and, indeed, in the overall food chain. Single cell phytoplankton often multiply by cell division and depend on photosynthesis as their main energy source.

Phytoplankton have been cultured in the laboratory for decades. They are often considered somewhat delicate or difficult to culture, but a variety of techniques, methods, and media have been developed which allow fairly routine culture of these organisms. There is a national facility for the preservation and culture of marine phytoplankton which stocks and maintains standard cultures of various marine phytoplankton for distribution to scientists and researchers. Common wisdom is that these organisms require exposure to atmospheric carbon dioxide and a saline water environment, with appropriate metal and vitamin nutrients.

In the laboratory, these organisms are maintained in transparent glass or plastic bottles wherein the organisms are continuously exposed to the atmosphere to allow CO_2/O_2 exchange or are regularly unsealed to allow such exchange. Typically, a container would be infused with CO_2 to ensure its adequate supply to these generally-regarded delicate organisms.

They are grown and studied by scientists. They are of interest as an indicator of productivity in the oceans and possibly even as an indicator of global warming and the greenhouse effect.

Phytoplankton are used in aquaculture installations and public aquaria as food for crustaceans, copepods, and zooplankton.

We have studied marine phytoplankton (dinoflagellates) which are bioluminescent. They produce light at night when mechanically stimulated. Our studies on these organisms initially employed the conditions which research scientists and experts had defined as optimum for culture and growth.

In using cultures of these organisms to demonstrate the process of bioluminescence to the general public, to teachers, and students, we had to expose the organisms to less than ideal environments. 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 any possibility of spillage. In some cases they had to be 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.

SUMMARY OF THE INVENTION

It has been discovered that containment of cultures of phytoplankton in envelopes of a translucent, gas-permeable, synthetic polymer film enables the cultures to be stored for extended periods of time requiring only periodic exposure to light. The gas-permeable film permits release of oxygen from the container and the infusion of carbon dioxide.

Gas permeable polymeric membranes useful in this invention include polyethylene, ethylene vinyl acetate copolymers, fluorinated ethylene propylene, copolymer, silicone rubber and microporous polymers. Such polymers are generally flexible, although rigid polymers may be utilized provided that they are translucent and gas-permeable.

Microorganisms which can be stored and shipped in translucent, gas-permeable envelopes include dinoflagellates, diatoms, algae and the like. Particular dinoflagellates which are susceptible to storage and shipment in accordance with this invention include Pyrocystis lunula, Pyrocystis noctiluca, and Pyrocystis fusiformis.

The scientific literature on the culture of these organisms generally cautions that they are delicate and require optimum laboratory conditions in order to successfully grow. Those skilled in the art advise that such organisms would not grow in plastic bag environments.

We have therefore discovered and applied a novel, highly practical, and convenient means to culture phytoplankton in sealed environments.

DETAILED DESCRIPTION OF THE INVENTION

We discovered that cultures of certain dinoflagellates could exist for over 12 months or more in a completely sealed environment provided that CO_2/O_2 photosynthesis and respiration could occur and that adequate periodic exposure to light occurred. The cultures develop a balance between photosynthesis and respiration, which allows them to reach a partial steady state and to survive in small sealed volumes for over a year.

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. Nevertheless, the discovery is that organisms that were considered frail, delicate, and difficult to culture have been shown to be hardy, tolerant, and capable of survival in situations and environments which were considered intolerable by those skilled in the art, provided that proper conditions, as stated hereinafter, are observed, especially that of a translucent, gas-permeable (O_2/CO_2) container.

The ability of phytoplankton to grow in sealed environments opens a wide range of possible applications, including the development of living specimens for science education, for environmental studies, and as detectors and sensors for various agents.

The cultures require moderate temperatures ranging from about 7°C to about 25°C, moderate light levels for 6-14 hours a day, and moderate handling; mechanical activation or stimulation should not be excessive.

These cultures are useful in science kits, as novelty products or the like, particularly in the case of bioluminescent organisms. They are also of interest as products and exhibits in marine aquaria, science centers, and museums. They are also applicable as living foods for the hobbyist aquarium and the aquaculture business.

Important variables affecting the longevity of a sealed phytoplankton culture include initial cell density, air volume to liquid volume ratio in the container, initial CO_2 level and pH, transparency of the container, initial composition of the seawater medium, mechanical stimulation and disturbances during the course of the culture, temperature, light intensity and light duration, details of the day/night cycle, the specific phytoplankton involved, bacteria and other organisms present in the culture, the nature of the material housing the culture (the flask or the bag), gas transport characteristics of that material, water

transport characteristics of that material, the particular chemical composition of the material from which the container is made, especially with respect to the ability of the organisms to attach or not attach to the walls of the container, and whether or not the container releases or binds chemicals to or from the culture medium.

Operational parameters for each of the above criteria are as follows:

Cell densities less than 5000 cells/ml, air volume to liquid volume about 1:3 to about 2:1, normal culture medium pH (~8.0-8.8), transparent, standard phytoplankton culture media, minimum mechanical stimulation, a temperature range of 7° to 25°C, a light/dark cycle of about 6:18 to 8:6, and, low density polyethylene (1 to 4 mil in thickness).

Preferred parameters for each of the above criteria are:

Cell density of about 500 cells/ml; an air/media volume ratio of about 1:1; pH 8.4-8.6; temperature of about 15°C; 12:12 light/dark cycle and, 2 mil thick polymer film having the desired translucency gas (CO_2/O_2) transport characteristics and a generally non-adherent, non-toxic surface.

Variations in the above-identified criteria may readily be experienced. For example, containers having a low gas diffusion rate may be used with lower concentrations of the microorganisms or by allowing a greater void space in the container, which may be filled with CO_2. Also, in such an instance, the translucency of the container may be altered so that less photosynthesis takes place. Thus, while the above-stated variables may affect longevity of storage, those skilled in the art will recognize from the above-recited variables and from the general description of

the invention, the types of adjustments to be made to achieve optimum conditions.

Microorganisms susceptible of being stored in accordance with the instant invention include marine dinoflagellates such as Pyrocystis lunula, Pyrocystis noctiluca and Pyrocystis fusiformis, diatoms and various algae. The common characteristics of such storage-stable micro-organisms are that they conduct sufficient photosynthesis to survive even when exposed to light only on an intermittent basis so long as a source of CO_2 is present.

Although flexible polyethylene containers have been particularly useful for the purposes of the instant invention, it should be understood that those polymers having similar characteristics of light transmission and oxygen/carbon dioxide diffusion may be useful, especially such polymers which are essentially inert with respect to the aqueous media and culture which are to be contained. Examples of other useful polymers include silicone rubbers, ethylene vinyl acetate polymers, fluorinated ethylene propylene copolymers, other fluorinated or partially fluorinated polymers, and microporous polymers.

Many small individual containers of cultures could be shipped in a larger protective container, e.g. a rigid container having porous sidewalls and internal structure providing open compartments for the individual containers. The porous sidewalls, which may be provided by having numerous small apertures in a rigid sidewall, would allow for passage of gasses to and from the individual culture containers. A battery operated light source, continuous or intermittent, could be included in the protective container to ensure proper light intensity during any pre-shipment and post-shipment storage as well as during shipment.

Alternatively, the protective container may be made from a translucent or transparent plastic so that a

light source was not required if storage was in a periodically lighted environment and shipment was of a short duration.

The protective containers may be made to accommodate one or just a few culture containers or to hold a large number of the individual containers.

The external protective containers may be useful especially for shipping purposes, inasmuch as the individual containers are generally "soft sided," being made preferably from flexible polymers.

Individual containers which are rigid plastics may be utilized if the proper gas transport properties exist. The plastic and glass containers used in the prior art storage of cultures did not have gas-permeable characteristics. Generally, the preferred polymeric films for best gas transport characteristics are flexible polymers, especially of the types identified hereinabove.

Example

A six-inch length of standard 2 mil thick, 2 inches wide, low density polyethylene tubing is cut to a six-inch length and heat sealed at one end using a common film heat sealer. The bag is then held vertically and approximately 30 cc's of marine phytoplankton suspension is dispensed into the bag. The top of the bag is then heat sealed such that the internal contents contains about 30 cc of the aqueous cell suspension and an additional 30 cc of air. Such a bag is completely sealed and is treated as a typical flask culture is treated, given light under normal culture illumination conditions for 6-14 hours per day. The same procedure can be used for bags of very different sizes ranging from one-centimeter tubes to twelve-inch tubes and ranging in length from 2 cm to 2 ft, together with comparable volumes.

Polyethylene thicknesses from 0.5 to 10 mil are appropriate, although 1 to 5 mil is preferred, while

especially useful thicknesses are from 2 to 4 mil. Depending upon the strength of the polymeric film, sidewalls as thin as 0.01 mil may be used. The use of thin films, consistent with strength requirements of the envelope, is generally preferred inasmuch as gas-diffusion, for a particular polymer composition, will occur more rapidly through thin films.

The invention described herein has been primarily with regard to storing and shipping cultures of marine microorganisms. While tests have not been conducted with fresh water organisms, it is anticipated that the invention may be readily applied to such microorganisms with useful results.

CLAIMS

What is claimed is:

1. A storage-stable article comprising:
envelope means of a material which is at least translucent and capable of oxygen and carbon dioxide diffusion through said envelope; and
an aqueous media containing a phytoplankton culture, said media contained within said envelope.

2. The article of claim 1, wherein said envelope means is a flexible polymeric material.

3. The article of claim 2, wherein said polymer is polyethylene.

4. The article of claim 2, wherein said polymeric material is transparent.

5. The article of claim 1, wherein said phytoplankton is a bioluminescent phytoplankton.

6. The article of claim 1, wherein the density of said phytoplankton in said aqueous medium is about 100 cells/ml to about 5000 cells/ml.

7. The article of claim 1, wherein said aqueous medium has a pH range of about 8.0 to about 9.0.

8. The article of claim 1, wherein said aqueous medium is a saline solution.

9. The article of claim 1, wherein said envelope has a wall thickness of about 0.01 to about 10 millimeters.

10. The article of claim 1, wherein said envelope means is only partially filled with media.

11. A method of storing a culture of live sea-originating microorganism comprising:
placing said microorganism in saline aqueous medium;
adjusting the pH of said aqueous medium to a range of about 8.4 to about 8.8;
placing the culture-containing aqueous medium into a container having thin walls which are at least translucent and through which oxygen and CO_2 diffuse;
sealing said container;
exposing said container to light with sufficient regularity to maintain a sufficient level of photosynthesis to sustain the microorganism in a living condition; and
maintaining the container at a temperature of about 7 to about 25°C.

12. The method of claim 11, wherein said microorganism in phytoplankton.

13. The method of claim 12, wherein said phytoplankton is bioluminescent.

14. The method of claim 11, wherein said phytoplankton is a sea-originating phytoplankton associated with other unidentified sea-originating microorganisms.

ABSTRACT

A culture of marine phytoplankton contained in transparent or semi transparent, gas permeable containers with a medium of natural or artificial sea water is disclosed. Such cultures are maintained indefinitely under appropriate light and temperature conditions.

Containment in polyethylene or other plastic bags enables the cultures to be easily handled, to be directly observed without direct contact, and amenable to a wide variety of scientific, technical, education, and entertainment purposes.

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FILING RECEIPT

UNITED STATES DEPARTMENT OF COMMERCE
Patent and Trademark Office
ASSISTANT SECRETARY AND COMMISSIONER
OF PATENTS AND TRADEMARKS
Washington, D.C. 20231

APPLICATION NUMBER	FILING DATE	GRP ART UNIT	FIL FEE REC'D	ATTORNEY DOCKET NO.	DRWGS	TOT CL	IND CL
08/056,168	04/30/93	2404	$420.00	2249	0	14	2

WILLIAM S. BRITT
TRASK, BRITT & ROSSA
P.O. BOX 2550
SALT LAKE CITY, UT 84110

Receipt is acknowledged of this patent application. It will be considered in its order and you will be notified as to the results of the examination. Be sure to provide the U.S. APPLICATION NUMBER, FILING DATE, NAME OF APPLICANT, and TITLE OF INVENTION when inquiring about this application. Fees transmitted by check or draft are subject to collection. Please verify the accuracy of the data presented on this receipt. If an error is noted on this Filing Receipt, please write to the Application Processing Division's Customer Correction Branch within 10 days of receipt. Please provide a copy of the Filing Receipt with the changes noted thereon.

Applicant(s) JOSEPH D. ANDRADE, SALT LAKE CITY, UT; JOHN D. TOBLER JR., SALT LAKE CITY, UT.

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TITLE
CULTURE, MAINTENANCE, STORAGE, AND TRANSPORT OF PHYTOPLANKTON IN SEALED ENVIRONMENTS

PRELIMINARY CLASS: 206

SEP 30

(see reverse)

Joe Andrade

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February 2, 1994

Mr. Tom Major, Director
University of Utah
Technology Transfer Office
421 Wakara Way, Suite 170
Salt Lake City, UT 84108

Re: U.S. PATENT APPLICATION

Invention	CULTURE, MAINTENANCE, STORAGE, AND TRANSPORT OF PHYTOPLANKTON IN SEALED ENVIRONMENTS
Inventors	Joseph D. Andrade et al.
Applicant/Assignee	University of Utah
Serial No.	08/056,168
Filing Date	April 30, 1993
Your Reference No.	U-1826
Our Case No.	2249

Dear Tom:

The convention year for filing foreign patent applications corresponding to the above-identified U.S. application will expire on April 30, 1994. If you wish to file convention applications in any foreign countries, we should have your instructions very soon.

In view of my recent discussion with Joe, I'm sure that you don't want to foreign file, however, our procedures require that this be sent.

Very truly yours,

William S. Britt

WSB/le

(check one)

☐ Yes, I wish to file convention applications in foreign countries. I will contact your office within the next month to obtain more information.

☒ No, I do not wish to file convention applications in any foreign countries.

Name:
Date: 2/3/94

C:\LETTERS\1Q94\2249.LTR(LE_)02/02/94

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October 27, 1993

SENT BY CERTIFIED MAIL

Mr. Tom Major, Director
University of Utah
Technology Transfer Office
421 Wakara Way
Suite 170
Salt Lake City, UT 84108

Re: U.S. PATENT APPLICATION

Invention	CULTURE, MAINTENANCE, STORAGE, AND TRANSPORT OF PHYTOPLANKTON IN SEALED ENVIRONMENTS
Inventors	Joseph D. Andrade and John D. Tobler, Jr.
Applicant/Assignee	University of Utah
Serial No.	08/056,168
Filing Date	April 30, 1993
Your Reference No.	U-1826
Our Case No.	2249

Dear Tom:

Enclosed is the original Assignment which has been recorded and returned by the Patent and Trademark Office in connection with the above-referenced application. You will note that the Assignment was recorded on July 2, 1993, reel 6672, frames 974 through 978. Also enclosed is a copy of the Official Notice of Recordation.

The assignment is like a deed and should be stored in a secure location.

We have filed the UURF assignment with the Patent and Trademark Office today.

Very truly yours,

Allen C. Turner

ACT/le
Enclosure: Original Assignment
Official Notice of Recordation
C:\LETTERS\4093\2249.LT(LE_) 10/27/93

UNITED STATES DEPARTMENT OF COMMERCE
Patent and Trademark Office
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Washington, D.C. 20231

DATE: 10/04/93
TO:

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WILLIAM S. BRITT
TRASK BRITT & ROSSA
P.O. BOX 2550
SALT LAKE CITY, UT 84110

UNITED STATES PATENT AND TRADEMARK OFFICE
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PLEASE REVIEW ALL INFORMATION CONTAINED ON THIS NOTICE. THE INFORMATION CONTAINED ON THIS RECORDATION NOTICE REFLECTS THE DATA PRESENT IN THE PATENT ASSIGNMENT PROCESSING SYSTEM. IF YOU SHOULD FIND ANY ERRORS OR QUESTIONS CONCERNING THIS NOTICE, YOU MAY CONTACT THE EMPLOYEE WHOSE NAME APPEARS ON THIS NOTICE AT 703-308-9723. PLEASE SEND REQUEST FOR CORRECTION TO: U.S. PATENT AND TRADEMARK OFFICE, ASSIGNMENT BRANCH, NORTH TOWER BUILDING, SUITE 10C35, WASHINGTON, D.C. 20231

ASSIGNOR: DOC DATE: 07/01/93
ANDRADE, JOSEPH D.

ASSIGNOR: DOC DATE: 07/02/93
TOBLER, JOHN D.

RECORDATION DATE: 07/02/93 NUMBER OF PAGES 005 REEL/FRAME 6672/0974

DIGEST : ASSIGNMENT OF ASSIGNORS INTEREST

ASSIGNEE:
MAJOR, TOM, DIRECTOR
TECHNOLOGY TRANSFER OFFICE
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SUITE 170
SALT LAKE CITY, UTAH 84108

SERIAL NUMBER 8-056168 FILING DATE 04/30/93
PATENT NUMBER ISSUE DATE 00/00/00

Tom Clark

EXAMINER/PARALEGAL
ASSIGNMENT BRANCH
ASSIGNMENT/CERTIFICATION SERVICES DIVISION

MT 25

$40.00 1581 A/D

JUL 12 1993 PAT. & TRADEMARK OFF.

RECORDATION FORM COVER SHEET
PATENTS ONLY

To the Honorable Commissioner of Patents and Trademarks: Please record the attached original documents or copy thereof.
Unless filed with a new application, mail to: BOX ASSIGNMENT, Commissioner of Patents and Trademarks, Washington, D.C. 20231.

1. Name of conveying party(ies):

Joseph D. Andrade
John D. Tobler

Additional name(s) of conveying party(ies) attached? ☐ Yes ☒ No

2. Name and address of receiving party(ies):

Name: Tom Major, Director
Technology Transfer Office
421 Wakara Way
Suite 170
City: Salt Lake City State: UT ZIP: 84108

Additional name(s) & address(es) attached? ☐ Yes ☒ No

RECEIVED
JUL 30 1993

3. Nature of conveyance:

☒ Assignment ☐ Merger
☐ Security Agreement ☐ Change of Name
☐ Other _____

Execution Date: July 1, 1993; July 2, 1993

4. Application number(s) or patent number(s):

If this document is being filed together with a new application, the execution date of the application is: _____

A. U.S. Patent Application No.(s)
08/056,168

B. U.S. Patent No.(s)

Additional numbers attached? ☐ Yes ☒ No

5. Name and address of party to whom correspondence concerning this document should be mailed:

Name: William S. Britt
Trask, Britt & Rossa
P.O. Box 2550
Salt Lake City, UT 84110

080 RJ 07/26/93 08056168 1 581

Attorney Docket No. 2249

6. Total number of U.S. applications and U.S. patents involved: 1

7. Total fee (37 CFR 3.41) .... $ 40.00
($40.00 times number in box 6)
Check no. 04009 is enclosed in this amount.

40.00 CK

8. The Commissioner is hereby authorized to charge any deficiency or credit any overpayment to deposit account number 20-1469.

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9. Statement and signature.
To the best of my knowledge and belief, the foregoing information is true and correct and any attached copy is a true copy of the original document.

William S. Britt — Name of Person Signing
Signature
7/2/93 — Date

Reg. No. 20,969

Total number of pages comprising cover sheet: 5

N:\TORSIS\COVER.SHT

ASSIGNMENT

In consideration of One Dollar ($1.00), and other good and valuable consideration including such remuneration as provided for by the University of Utah's patent policy 6-4 regarding sharing of revenues from licensing, the receipt of which is hereby acknowledged, We, the undersigned, Joseph D. Andrade and John D. Tobler, Jr. ("ASSIGNOR"),

Hereby sell, assign and transfer to the University of Utah ("ASSIGNEE"), a non-profit organization existing under the laws of the State of Utah, its successors, assigns and legal representatives, the entire right, title and interest for the United States and all foreign countries, including priority rights, in and to any and all improvements for which an application for United States Letters Patent has been executed by the undersigned ASSIGNOR concurrently herewith, and is entitled,

CULTURE, MAINTENANCE, STORAGE, AND TRANSPORT OF PHYTOPLANKTON IN SEALED ENVIRONMENTS

has been filed under Serial No. 08/056,168, on April 30, 1993, and in and to said application and all divisional, continuing, substitute, renewal, reissue, and all other applications for Letters Patent which have been or shall be filed in the United States and all foreign countries on any of said improvements; and in and to all original and reissued patents which have been or shall be issued in the United States and all foreign countries on said improvements;

Agree that said ASSIGNEE may apply for and receive Letters Patent for said improvements in its own name; and that, when requested, without charge to but at the expense of said ASSIGNEE, its successors, assigns and legal representatives, to carry out in good faith the intent and purpose of this assignment, the undersigned will execute all divisional, continuing, substitute, renewal, reissue, and all other patent applications on any and all said improvements; execute all rightful oaths, assignments, powers of attorney and other papers; communicate to said ASSIGNEE, its successors, assigns, and representatives, all facts known to the undersigned relating to said improvements and the history thereof; and generally do everything possible which said ASSIGNEE, its successors, assigns or representatives shall consider desirable for aiding in securing and maintaining proper patent protection for said improvements and for vesting title to said improvements and all applications for patents and all patents on said improvements, in said ASSIGNEE, its successors, assigns and legal representatives; and

Covenant with said ASSIGNEE, its successors, assigns and legal representatives that no assignment, grant, mortgage, license or other agreement affecting the rights and property herein conveyed has been made to others by the undersigned,

and that full right to convey the same as herein expressed is possessed by the undersigned.

ASSIGNOR:

(Signature) _____ Date: 7/1/93

Name: Joseph D. Andrade

STATE OF UTAH)
: ss.
County of Salt Lake)

Before me this 1st day of July, 1993, personally appeared Joseph D. Andrade, the person who is described in and who executed the above instrument, and acknowledged to me that he executed the same of his own free will for the purpose therein set forth.

DARLENE HOLT
Notary Public
STATE OF UTAH
My Comm. Expires OCT 27, 1996
525 S 3RD E SLC UT 84111

_____
Notary Public
Residing at:

My Commission Expires:
Oct 27, 1996

ASSIGNOR:

(Signature) _____ Date: 2 July 93
Name: John D. Tobler, Jr.

STATE OF UTAH)
: ss.
County of Salt Lake)

Before me this 2nd day of July, 1993, personally appeared John D. Tobler, Jr., the person who is described in and who executed the above instrument, and acknowledged to me that he executed the same of his own free will for the purpose therein set forth.

DARLENE HOLT
Notary Public
STATE OF UTAH
My Comm. Expires OCT 27, 1996
525 S 3RD E SLC UT 84111

Darlene Holt
Notary Public
Residing at:

My Commission Expires:
Oct 27, 1996

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TRADEMARK OFFICE
JUL -2 93

Law Offices
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May 4, 1994

Mr. Tom Major, Director
University of Utah
Technology Transfer Office
421 Wakara Way, Suite 170
Salt Lake City, UT 84108

Re: U.S. PATENT APPLICATION

Invention	CULTURE, MAINTENANCE, STORAGE, AND TRANSPORT OF PHYTOPLANKTON IN SEALED ENVIRONMENTS
Inventors	Joseph D. Andrade et al.
Applicant/Assignee	University of Utah
Serial No.	08/056,168
Filing Date	April 30, 1993
Your Reference No.	U-1826
Our Case No.	2249

Dear Tom:

After discussion with Joe Andrade and review of the cited art, it has been decided to let the above-identified patent application go abandoned.

We will also reduce the patent application charge by $500.00, as per my conversation with Joe Andrade.

If you have any questions, please feel free to call.

Very truly yours,

William S. Britt

WSB/le

cc: Joseph Andrade

C:\LETTERS\2Q94\2249(LE_) 05/04/94

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June 8, 1994

Mr. Tom Major, Director
University of Utah
Technology Transfer Office
421 Wakara Way, Suite 170
Salt Lake City, UT 84108

Re: U.S. PATENT APPLICATION

Invention	CULTURE, MAINTENANCE, STORAGE, AND TRANSPORT OF PHYTOPLANKTON IN SEALED ENVIRONMENTS
Inventors	Joseph D. Andrade et al.
Applicant/Assignee	University of Utah
Serial No.	08/056,168
Filing Date	April 30, 1993
Your Reference No.	U-1826
Our Case No.	2249

Dear Tom:

This letter confirms that the above-identified application has become abandoned. Enclosed is a copy of the formal Notice of Abandonment. It is sometimes possible to revive an abandoned application. If you wish to revive this application, the appropriate papers should be prepared and filed along with the required fees as soon as possible.

After a year from the date of abandonment or May 26, 1995, it becomes exceedingly difficult to revive an abandoned application.

If you have any questions, please contact me.

Very truly yours,

William S. Britt

WSB/le
Enclosure: Copy of Formal Notice of Abandonment
cc: Joseph Andrade
C:\LETTERS\2Q94\2249(LE_)06/08/94

UNITED STATES DEPARTMENT OF COMMERCE
Patent and Trademark Office

Address : COMMISSIONER OF PATENTS AND TRADEMARKS
Washington, D.C. 20231

SERIAL NUMBER	FILING DATE	FIRST NAMED APPLICANT	ATTORNEY DOCKET NO.
08/085,163	06/30/93	ANDRADE, J	2249

WILLIAM A. BRITT
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SALT LAKE CITY, UT 84110

EXAMINER	
ART UNIT	**PAPER NUMBER**
1808	5

DATE MAILED: 05/26/94

NOTICE OF ABANDONMENT

This application is abandoned in view of:

1. ☒ ~~Applicant's failure to respond to the Office letter, mailed~~ 10/20/93 .
2. ☐ Applicant's letter of express abandonment which is in compliance with 37 C.F.R. 1.138.
3. ☐ Applicant's failure to timely file the response received _____ within the period set in the Office letter.
4. ☐ Applicant's failure to pay the required issue fee within the statutory period of 3 months from the mailing date of _____ of the Notice of Allowance.

   ☐ The issue fee was received on _____ .

   ☐ The issue fee has not been received in Allowed Files Branch as of _____ .

   In accordance with 35 U.S.C. 151, and under the provisions of 37 C.F.R. 1.316(b), applicant(s) may petition the Commissioner to accept the delayed payment of the issue fee if the delay in payment was unavoidable. The petition must be accompanied by the issue fee, unless it has been previously submitted, in the amount specified by 37 C.F.R. 1.17 (l), and a verified showing as to the causes of the delay.

   If applicant(s) never received the Notice of Allowance, a petition for a new Notice of Allowance and withdrawal of the holding of abandonment may be appropriate in view of Delgar Inc. v. Schuyler, 172 U.S.P.Q. 513.

5. ☐ Applicant's failure to timely correct the drawings and/or submit new or substitute formal drawings by _____ as required in the last Office action.

   ☐ The corrected and/or substitute drawings were received on _____ .

6. ☐ The reason(s) below.

June 1994

DOUGLAS W. ROBINSON
SUPERVISORY PATENT EXAMINER
ART UNIT 188

PTO-1432 (REV. 5-83)