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Abstracts of Presentations Aquatic Species Program Annual Review Meeting

Solar Energy Research Institute
Golden, Colorado

September 24-25, 1986

Solar Energy Research Institute
A Division of the Midwest Research Institute

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FINAL AGENDA

AQUATIC SPECIES PROGRAM
ANNUAL REVIEW MEETING

SOLAR ENERGY RESEARCH INSTITUTE

September 24-25, 1986
Golden, Colorado

Wednesday, September 24

8:00 - 8:20 am	Registration	Coffee, Danish Bldg. 17 4th Floor, Room 4B
PROGRAM REVIEW		
8:20 - 8:25 am	Welcome	Stanley Bull SERI
8:25 - 8:45 am	DOE Perspective	Sarah Sprague DOE
8:45 - 9:15 am	Program Overview	Donna Johnson SERI
SESSION I: SPECIES SCREENING		
9:15 - 9:35 am	Screening Overview: Protocol Modification and Evaluation	Bill Barclay SERI
9:35 - 9:55 am	Isolation and Screening of Oleaginous Microalgae	Keith Cooksey Montana State University
9:55 - 10:15 am	Species from the Desert Southwest	Milton Sommerfeld Arizona State University
10:15 - 10:30 am	BREAK	
10:30 - 10:50 am	Species from the Southeastern U.S.	Mahasin Tadros Alabama A&M
10:50 - 11:10 am	Species from the Hawaiian Islands	Richard York Hawaii Inst. of Marine Biology
11:10 - 11:30 am	Oil Production by Picoplankton	Ralph Lewin Scripps Institute of Oceanography

11:30 - 11:50 am	Physiological Variability in Strains of Chaetoceros muelleri	Jeff Johansen SERI
11:50 am - 1:30 pm	LUNCH	Bldg. 15 4th Floor

SESSION II: OUTDOOR MASS CULTURE RESEARCH

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2:00 - 2:30 pm	Integrated Microalgal Lipid Production	Shoshana Arad Ben-Gurion University
2:30 - 3:00 pm	Harvesting and Lipid Extraction	G. Shelef Technion, Israel Institute of Technology
3:00 - 3:15 pm	BREAK	
3:15 - 3:45 pm	Productivity Optimization of Saline-Adapted Microalgae Grown in Outdoor Mass Culture	Ed Laws University of Hawaii

SESSION III: SALINE WATER RESOURCES

3:45 - 4:15 pm	New Mexico Saline Water Resources	Robert Lansford New Mexico State University
4:15 - 4:35 pm	Arizona Saline Water Resources	Mike Osborn University of Arizona
4:35 pm	ADJOURN	
4:35 - 5:30 pm	Discussion with Program Advisors and Session I, II and III Presentors	
5:30 - 6:30 pm	Reception, Cash Bar	Marriott Hotel
6:30 - 8:30 pm	Banquet	Marriott Hotel

Thursday, September 25

8:00 - 8:20 am	Registration	Coffee, Danish Bldg. 17 4th Floor, Room 4B

SESSION IV: PHYSIOLOGY, BIOCHEMISTRY, GENETICS

8:20 - 8:50 am	Nutritional Requirements of Oil-Producing Microalgae	Charles Rhyne Jackson State University

8:50 - 9:10 am	Environmental Control of Lipid Induction	Paulanne Chelf SERI
9:10 - 9:30 am	Effects of Light Intensity on Lipid Induction	Nick Nagle SERI
9:30 - 10:00 am	Flow Cytometry Techniques for Species Improvement	Jean Soloman Oak Ridge National Lab.
10:00 - 10:30 am	Biochemical Elucidation of Neutral Lipid Synthesis	Keith Cooksey Montana State University
10:30 - 10:45 am	BREAK	
10:45 - 11:15 am	Biochemistry of Neutral Lipid Synthesis in Microalgae	Stephen Schwartzbach University of Nebraska
11:15 - 11:45 am	Biochemical Aspects of Lipid Accumulation in Silicon-Deficient Diatoms	Paul Roessler SERI
11:45 am - 12:15 pm	Chemical Characterization of Microalgal Lipids	Shobba Sriharan Selma University
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SESSION IV CONT: PHYSIOLOGY, BIOCHEMISTRY, GENETICS

1:30 - 2:00 pm	Effects of Fluctuating Environments on the Selection of High-Yielding Microalgae	John Benemann Georgia Institute of Technology
2:00 - 2:30 pm	Temperature Effects on Microalgal Photosynthetic Efficiency	Paul Behrens Martek Corporation
2:30 - 3:00 pm	Genetic Variation in Oil-Producing Microalgae	Jane Gallagher City College of New York
3:00 - 3:15 pm	BREAK	
3:15 - 3:45 pm	Kelp Genetics	Michael Neushul Neushul Mariculture Inc.
3:45 - 4:14 pm	Characterization of Viruses Infecting Chlorella-Like Alga	Richard Meints University of Nebraska
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SESSION IV: PHYSIOLOGY, BIOCHEMISTRY, GENETICS

AQUATIC SPECIES PROGRAM OVERVIEW

Donna A. Johnson
Solar Energy Research Institute, Golden, CO 80403

The goal of the Aquatic Species Program is to produce gasoline and diesel fuels from microalgae grown in saline waters of the desert Southwest. Microalgae are known to accumulate lipids in large quantities and can thrive in high salinity water which currently has no other use. Three major task areas are important to the economical development of this technology; biology, engineering, and analysis.

Biological activities include screening, characterization and improvement of microalgae species. Extensive efforts began in 1982 to collect and screen microalgae strains which are salinity and temperature tolerant, highly productive, and which produce large amounts of lipids. Over 3,000 microalgae strains have been collected to date. Species used by the program in 1982 had temperature tolerances of 15-25°C and salinity tolerances from 20-40 mmho cm^{-1}. With the intensive collection efforts, the program now has strains which can tolerate wide environmental fluctuations, from 10-35°C and 10-70 mmho cm^{-1}. Rates of productivity have increased from 10-20 g dry wt m^{-2} day^{-1} in 1982 to greater than 50 g dry wt m^{-2} day^{-1} under laboratory conditions and over 35 g dry wt m^{-2} day^{-1} in outdoor systems in 1986. There have also been significant increases in lipid content of the algal cells from 20% in 1982 to 66% indoors and 40% in outdoor culture by 1986. One of the current problems is that species which are salinity and temperature tolerant do not always have high productivity and/or produce large amounts of lipid. Therefore, basic research is currently underway in genetic engineering to put all three characteristics into one or two strains.

Engineering research focused on polymer harvesting of microalgae. All algae were harvestable but required different polymers. Harvesting was accomplished for 0.5-1.5¢/kg dry mass, with removal efficencies greater than 85-95%. Cross-flow microfiltration was tested and determined to be too costly. Another method of harvesting examined was flocculation. Recycling flocculants reduced costs by 100-200%.

A technical and economic analysis, "Fuels from Microalgae," has been published which demonstrates that gasoline and diesel fuels can be produced from mass-cultured microalgae at prices that will be competitive with conventional fuels. Aggressive research is needed but the improvements are within the bounds of attainability. A major choke point has been the availability of saline water resources in the desert Southwest. It has been demonstrated that there exists enough saline water in Arizona and New Mexico to produce at least one quad of energy from microalgae.

Future activities of the Aquatics Program include: completing collection activities and focusing on characterizing species collected; consolidating all outdoor test facilities into one large, 0.5 - 1.5 ha, system in a desert region; harvesting and carbon dioxide supply research; and converting microalgae lipids into liquid fuels. Research will continue on algal physiology, biochemistry and genetic engineering.

SESSION I: Species Screening

Wednesday, September 24, 1986
9:15 - 11:50 a.m.

SCREENING MICROALGAE FOR BIOMASS PRODUCTION POTENTIAL: PROTOCOL MODIFICATION AND EVALUATION

B. Barclay, N. Nagle, and K. Terry
Solar Energy Research Institute

Our laboratory developed and tested a collection and screening protocol to isolate microalgal strains for biomass production applications. The protocol involved a process for exposing nutrient enriched collection site waters (from shallow, inland saline habitats) to high light intensities (20-50% of sunlight) and elevated temperature (30°C) on a rotary screening apparatus. The purpose of the protocol was to select for strains with fast growth rates that could tolerate the high light intensities and temperatures anticipated in mass cultivation systems in the southwestern U.S.

This protocol has proven successful in identifying several excellent strains which exhibit fast growth rates in combination with a wide range of salinity and temperature tolerance. The dominant genera isolated by the protocol include lipid producing species of Chaetoceros, Amphora, Monoraphidium, Nitzschia, Thalassiosira, and Navicula. The physiological attributes of several of these strains are reviewed and discussed in light of the chemical and physiological selection factors that appeared to be operating in the protocol. The effectiveness of an implemented improvement in the protocol is also discussed. Efforts to adapt the protocol to isolate cool-water (15°C) adapted strains are described.

Isolation and screening of oleaginous microalgal isolates. K. E. Cooksey, Department of Microbiology, Montana State University, Bozeman, MT, 59715.

One of the objectives of our research was to design a method to select algal strains producing large quantities of neutral lipid. For the purposes of this study, neutral lipid was defined as the triacylglyceride and hydrocarbon fraction of the cell. Since neither of these groups of compounds have functional groups that are easy to assay chemically, bulk lipid is often weighed after extraction from tissues with non-polar solvents or solvent mixtures. The method is time consuming and requires a sample large enough to produce a weighing (for the extracted lipid) in the milligram range. Greenspan and Fowler (1985) drew attention to the fact that Nile Red was fluorescent in non-polar but not in polar environments and pointed out that the stain could serve as a fluorescent lipid probe. We have extended this idea into a semiquantitative method for the determination of neutral lipid in small populations of single cells. The method has been developed using two types of fluorometer. In the first experiments we used a Becton-Dickinson FACS440 flow cytometer to measure the fluorescence of algal populations. In the second part of the work we have followed the time-course of fluorescence development of decay using a Spex fluorolog spectrofluorometer. Both machines gave linear relationships for fluorescence of Nile Red-stained cells and their lipid content. The fluorometric method is at least 100x more sensitive than the gravimetric method. The method we describe will facilitate screening of lipid-producing algae whether isolated from nature or produced by laboratory manipulation. A second objective of our research has been to isolate thermotolerant microalgae from thermal hot springs. The rationale here has been that such thermal areas should be naturally enriched in thermotolerant algae and thus the relative success in their isolation would be improved. All the algae isolated by so far grow well at 35^{o}C, a temperature considered close to the normal limit for mesophilic microalgae. Thermotolerant algae are expected to grow better in ponds in a desert environment where mid-day air temperatures will exceed 38^{o}C.

COLLECTION OF HIGH ENERGY YIELDING STRAINS OF SALINE MICROALGAE FROM SOUTHWESTERN STATES

Milton R. Sommerfeld and Stephen B. Ellingson
Department of Botany and Microbiology
Arizona State University, Tempe 85287

The primary goal of this research is to obtain high performance microalgal species from nature that may serve as the raw material required to make microalgae culture technology a plausible approach to fuel production. The specific objectives are to (1) collect strains of microalgae from a diversity of saline habitats in the desert Southwest, (2) isolate and identify strains that grow well at elevated salinities and light intensities and (3) characterize selected strains for lipid and carbohydrate accumulation. Microalgal and water samples were obtained from 103 sites in Arizona, California, Nevada, New Mexico, Texas, and Utah during 1985 and 1986. Collected waters ranged in temperature from 17.8 to 45.6 C, in specific conductance from 447 to 474,000 uS/cm and from 6.1 to 10.2 in pH. Ionic analysis of the water revealed that the relative anion and cation composition of the surface waters sampled was relatively similar to the artificial SERI Type I and Type II Media used as the standardized media for screening and growth rate experiments. The proportion of magnesium in SERI Type I Media, however, was higher than found in the waters sampled in the Southwest. Microalgae were isolated by either streaking directly onto agar plates of SERI Media or screened on a rotary culture device at elevated temperature and light intensity before streaking onto agar plates. Approximately 1600 individual isolates of microalgae were obtained from surface waters in the Southwest. Of the initial 57 algae screened for growth characteristics, the majority grew best at the lower salinities in both SERI Type I and II Media. Growth rates for 15 isolates exceeded one doubling/day and for five strains exceeded two doublings/day. In nutrient-sufficient batch cultures 14 strains yielded an average lipid concentration of 11.1% of ash-free dry weight. The highest yield was over 26%. Laboratory efforts are now being directed toward growth and chemical characterization of strains within specific taxonomic groupings such as the diatoms. Of 22 diatom strains that have demonstrated rapid growth, one-third of the strains exceeded one doubling/day and several gave positive indications of lipid accumulation. Future research will focus on evaluation of the effects of nitrogen and silicon deficiency and other growing conditions on lipid production in those strains exhibiting rapid growth.

SCREENING AND CHARACTERIZING OLEAGINOUS MICROALGAL SPECIES FROM THE SOUTHEASTERN UNITED STATES

Mahasin G. Tadros
Biology Department
Alabama A&M University
Normal, Alabama 35762

ABSTRACT

In recent years, interest in microalgal lipids has been renewed because of an urgent need for utilization of alternative renewable resources as carbon sources for the production of liquid fuels. Algal lipids are highly reduced hydrocarbons produced by direct conversion of solar energy to chemical energy via the process of photosynthesis. Microalgal species are capable of producing biomass yields containing high percentages of oils. The greatest challenge to developing and commercializing microalgae for the production of liquid fuels is the economical yield of a microalgal product. Among the factors which improve the yield of microalgal lipids are variations of environmental conditions. This ongoing project, sponsored by the Solar Energy Research Institute/Department of Energy under the Historically Black Colleges and Universities Program, is dealing with screening and optimizing microalgae for lipid production.

In the first year of this project (1984-1985), two diatoms: Cyclotella 35 and Nitzschia 160, which accumulated lipids up to 42% and 66% respectively under nitrogen stress, were included in the Aquatic Species Program of the Solar Research Energy Institute. During the second year of this project (1985-1986) two collection trips were made from the intertidal region and near islands along the coasts of Florida, Alabama and Mississippi. Among seventy five algal strains isolated, five fast growers and lipid accumulators were characterized: Navicula 260, Navicula 264, Nitzschia 225, Cylindrotheca 204, Chlorococcum 183. Three diatoms are of particular interest because of their high growth rate, tolerance and ability to accumulate high lipids. Navicula 260, and Navicula 264 cultures limited in nitrogen and silicon showed 34.6, 32.4, and 42.5% lipids respectively. However, Nitrogen-limited cells of Nitzschia 225 exhibited higher lipids (42.6%) than silica-limited cells (32.6%).

At present, the following species: Nitzschia 307, Navicula 304, Navicula 324 and Amphiprora 333, have been isolated and are under characterization.

COLLECTION OF HIGH ENERGY YIELDING STRAINS OF SALINE MICROALGAE FROM THE HAWAIIAN ISLANDS

Subcontract No. XK-04136-02

Richard H. York, Jr.
Hawaii Institute of Marine Biology
46-007 Lilipuna Road
Kaneohe, Hawaii 96744-1346

ABSTRACT

Microalgae were collected from 48 locations in the Hawaiian Islands in 1985. The sites were an aquaculture tank; a coral reef; bays; a geothermal steam vent; Hawaiian fish ponds; a Hawaiian salt punawai (well); the ocean; river mouths; saline lakes; saline pools; saline ponds; a saline swamp; and the ponds, drainage ditches and sumps of commercial shrimp farms. Conductivities of the water ranged from 6.00×10^2 micromhos cm^{-1} to 3.85×10^5 micromhos cm^{-1}, and temperatures ranged from 10.5°C to 62.0°C. Single cells or colonies of microalgae were isolated into media in glass culture tubes incubated in fluorescent light in the laboratory, and into fluorocarbon plastic bags transmitting full-spectrum sunlight outdoors.

From 4800 isolations, 100 of the most productive clones were tested for growth rate and photoinhibition in a solarium under conditions expected in production-scale facilities. One-half of the solarium had full-spectrum solar illumination at intensities greater than 80% of surface solar irradiance. The other half had the ultraviolet (UV) wavelengths blocked and the other wavelengths at intensities comparable to the full spectrum side. Temperatures were allowed limited increases during the day. All 100 clones were inoculated to approximately equal optical density at 750 nm and incubated simultaneously on each side of the solarium. On the full-spectrum side, the 50 fastest growing clones were Bacillariophytes, Chlorophytes, Cyanophytes, Pyrrophytes and unidentified flagellates. Twenty clones were selected for further study. Photoinhibition was an important factor in the selection process. Some of the clones with the highest growth rates without UV grew relatively slowly in full spectrum sunlight. Other clones showed no significant difference.

Five clones were tested for growth rate and production. The cultures were bubbled with carbon dioxide and air. Temperatures ranged from 17.7°C to 42.8°C. The highest growth rates were 2.12 doublings day^{-1} for Chaetoceros sp. clone SH 9-1, and 1.43 doublings day^{-1} for Cyclotella sp. clone SH 14-89. The highest production was 31 g dry weight $m^{-2} day^{-1}$ for the Chaetoceros and 33 g dry weight m^{-2} day^{-1} for the Cyclotella.

SESSION II: Outdoor Mass Culture Research

Wednesday, September 24, 1986
1:30 - 3:45 p.m.

OIL PRODUCTION BY PICOPLANKTON

RALPH A. LEWIN and LANNA CHENG

Scripps Institute of Oceanography, La Jolla, California

Picoplankton algae, 1-5 micrometers in diameter, include both prokaryotes and eukaryotes. Among the former (Cyanophytes), some can float by making gas vacuoles. Among the latter, some can swim by using flagella, others float because of stored oil. Nannochloropsis (Eustigmatophyta) produces a lot of oil, much of it hydrocarbon. By differential filtration and flotation enrichment cultures we have collected and examined the floating picoplankton of some 200 samples of inshore marine waters from subtropical and tropical sites, chiefly in the Caribbean. Pure cultures have been isolated and grown in defined media: their lipid production is being assessed under various conditions of culture. Some may ultimately prove of economic value as a source for liquid fuel.

Johansen, Jeffrey R. and William R. Barclay. Physiological variability in strains of Chaetoceros muelleri.

It is likely that at some time in the future, genetic engineering of selected algal strains will be performed to improve lipid production in strains isolated from the field. Before this work can be undertaken, the degree of genetic variability present in closely related algal strains, representing single species or species complexes, must be established. In order to assay genetic variability, we are currently comparing the physiology, morphology, and biochemistry of a number of strains of Chaetoceros muelleri isolated from various locations in the western United States. This species was chosen because representative strains are generally euryhaline, tolerant of high temperatures, rapid growing, and high in lipid content.

Presently, the growth response of three strains of C. muelleri to water type, conductivity, and temperature has been determined. Two strains, those from brackish waters in Utah and New Mexico, are very similar, both having optimal growth rates of over 3 doublings/day in SERI type II waters at temperatures between 25 and 35 C. Both strains are nearly euryhaline. These strains also appear morphologically identical. The third strain, from Lake Tuendae, California, is very different physiologically. It is slower growing, having optimal growth rates of only 1.7 doublings/day. It does poorly in SERI type II waters, growing best in SERI artificial seawater (GPM) media of low conductivity (10-25 mmhos/cm). It does very poorly or ceases to grow in all waters of high conductivity (55-70 mmhos/cm). Morphologically, the three strains are almost identical, with only one minor morphological difference having been noted so far. Based on morphology, all three are circumscribed by the description for Chaetoceros muelleri var. subsalsum.

These three strains demonstrate that within a single species, substantial genetic variability exists. We plan to continue our study of variability in C. muelleri and related species, having 38 strains currently in our culture collection from which to choose. We plan to characterize selected species in terms of: 1) growth response to water type, conductivity, and temperature, 2) growth response to light intensity, 3) lipid content, 4) sterol composition, 5) pigment ratios, and 6) enzymatic differences as determined by starch gel enzyme electrophoresis.

FACTORS AFFECTING THE PHOTOSYNTHETIC YIELD OF MICROALGAE

Joseph C. Weissman and Raymond P. Goebel
Microbial Products, Inc.
408A Union Avenue
Fairfield, CA 94533

ABSTRACT

The objectives of the subcontract to Microbial Products, Inc. include the outdoor cultivation of pre-screened algal species for an evaluation of productivity potential and competitiveness, development of harvesting technologies, engineering evaluation and design of photobioreactors, and physiological characterization of strains in the laboratory to assess factors which affect outdoor yield. There is substantial interplay among these objectives, especially the first and the last.

Twelve pre-screened species of algae were grown in 1.4 m^2 pond reactors, including six diatoms and six chlorophytes. Four of the diatoms (three strains of Chaetoceros and a Cyclotella) could be cultivated for as long as was attempted, from 28 to 125 days, at average productivities of about 30 $gm^{-2}d^{-1}$. The other two diatoms (Amphora sp. and Navicula sp.) were as productive but could not be sustained in culture. Productivity from the chlorophytes was generally only one-half to one-third as much as from the diatoms. Only species of one genus, Tetraselmis, endured in the outdoor culture tanks.

Factors which were found to affect both the yield and longevity of outdoor mass culture were photoinhibition, oxygen inhibition, temperature, pH, pCO_2, dilution rate, and nutrient status. Species which could not be sustained were often found to be unstable at high irradiance and/or high oxygen. Usually inability to adapt to the pond environment resulted quickly in the loss of a strain. In one case, predominance of Tetraselmis over Nannochloris, competition from another species determined the longevity of a culture. Changes in many of the variables listed above did result in productivity differences. However, the factor which determines the maximum yield is most likely the irradiance at which photosynthesis saturates. How to determine this and how to optimize it still remain elusive goals.

Integrated Microalgal Lipid Production

S. Arad
Ben-Gurion University

ABSTRACT NOT AVAILABLE AT TIME OF PRINT

Harvesting and Lipid Extraction

G. Shelef
Technion
Israel Institute of Technology

ABSTRACT NOT AVAILABLE AT TIME OF PRINT

PRODUCTIVITY OPTIMIZATION OF SALINE MICROALGAE GROWN IN OUTDOOR MASS CULTURE SYSTEMS

Dr. Edward Laws
University of Hawaii

Abstract

Optimization studies in a $50m^2$ outdoor flume using the marine diatom Cyclotella resulted in average photosynthetic efficiencies of 10% (based on visible light quanta) and production rates in excess of 30 g ash-free dry wt $m^{-2}d^{-1}$ over the course of a one year period. Two key features of the production system were the use of foil arrays to effect systematic vertical mixing of the culture and the use of batch culture growth intervals of two days. A year-long study of the effects of the foil arrays showed that production was enhanced by 25-30% with the foil arrays in place and spaced at intervals of four feet in the culture flume versus a conventional system with no foil arrays. A study of photosynthetic efficiency as a function of initial biomass density and the duration of the batch cycle revealed that (a) at initial densities below about 40 g AFDW m^{-3} production was highest when the cells were grown on a three-day cycle versus a one or two-day cycle (b) at initial densities above about 500 g AFDW m^{-3} production was highest when the cells were diluted daily (c) at initial densities between about 40 and 500 g AFDW m^{-3} the best production was achieved when the cells were diluted every two days (d) the best production, corresponding to a photosynthetic efficiency of about 10%, was achieved when the culture was diluted every two days.

SESSION III: Saline Water Resources

Wednesday, September 24, 1986
3:45 - 5:30 p.m.

ABSTRACT

EVALUATION OF AVAILABLE SALINE WATER RESOURCES IN NEW MEXICO FOR THE PRODUCTION OF MICROALGAE

by

Robert R. Lansford, John W. Hernandez, and Phillip Enis

The major objective of the research was the selection of potential site locations for 1000 hectares (2470 acres) microalgae production facilities in New Mexico using saline water resources. The emphasis of the research was twofold.

First, a data base was created with respect to the SERI criteria for location of microalgae production facilities in New Mexico. Specific criteria included location, depth, aquifer characteristics and saturated thickness of aquifers of interest; salinity, ionic composition and well-yields; and growing season, topography and land ownership.

Second, the data base was digitized for the construction of instructive maps. The desirable water supply for algae culture was limited to "moderately" or more saline groundwaters (3000 mg/l total dissolved solids or greater), because of the existing societal demands for the limited supply of better quality water.

After review of the 15 billion acre feet of saline water resources in the state, areas that appeared to meet all of the SERI criteria for site selection were narrowed to the following--the Tularosa Basin in south-central New Mexico, the Estancia Basin in central New Mexico, the San Juan Basin in northwestern New Mexico, the Tucumcari area in Quay County on the east side of the state, the area east of the Pecos River Basin in eastern New Mexico, and the Crow Flats area in southern New Mexico.

A detailed analysis was completed for the six locations. Three basins were eliminated for failing to meet all the criteria developed for the study--Pecos Basin, San Juan Basin and the Tucumcari Area. Of the remaining basins, the Tularosa was judged best suited for a microalgae production facility, the Crow Flats next best, and the Estancia the poorest of the three choices because of a short growing season.

Utilization of Saline Water Sources in Arizona for Microalgae Production

By:

Kevin L. Olson, Graduate Research Assistant, Water Resources Research Center, The University of Arizona, Tucson, AZ

L.G. Wilson, Hydrologist, Water Resources Research Center, The University of Arizona, Tucson, AZ

Mary Wallace, Research Assistant, Water Resources Research Center, University of Arizona, Tucson, AZ, and

M.D. Osborn, Research Assistant III, Water Resources Research Center, The University of Arizona, Tucson, AZ

ABSTRACT

Saline water sources are generally rejected for most uses. In contrast, the Solar Energy Research Institute (SERI) is examining the potential use of saline water sources for development of microalgae biomass systems for liquid fuel production. The development of such sources in the southwest is particularly attractive because of an abundance of flat land, high incident solar radiation, and saline water. The general purpose of a study undertaken by the Water Resources Research Center for SERI was to assess the potential of saline water sources in Arizona for development of a microalgae production facility. Specific objectives included delineating areas in the state with saline (i.e., TDS > 3000 mg/l) aquifers with pumping levels within 500 ft of land surface, and with the potential of yielding in excess of 4 mgd for up to 10 years; defining the chemical composition of the screened sources; and identifying possible legal, institutional and environmental constraints on a project.

Fifty five identified ground-water sources were screened. Of the 24 sources meeting the screening criteria, most are located within 4 regions in Arizona: along the Colorado River, along the upper and lower Gila River, near Casa Grande, and a large area on the Colorado Plateau in east-central Arizona. The majority of saline ground-water sources in Arizona have sodium and chloride as dominant ions. Surface water chemistry varies throughout the State, but in general, the water is dominated by sodium and chloride and/or magnesium and sulfate ions.

Legal-institutional considerations on microalgae production using saline ground waters include requirements for water rights acquisition under the 1980 Groundwater Management Act, potential problems arising from the ongoing Gila River adjudication, and current and impending legislation on ground-water quality.

SESSION IV: Physiology, Biochemistry, Genetics

Thursday, September 25, 1986
8:20 a.m. - 4:15 p.m.

Charles Rhyne
Department of Biology
Jackson State University
Jackson, MS 39217

TITLE:

Nutritional Requirements for Maximal Growth of Oil Producing Microalgae

SERI #5-05056

ABSTRACT

The purpose of this study was to define the nutritional requirements for selected known oil producing microalgae orginally from marine and inland saline habitats. Species tested were SERI collections of Ankistrodesmus falcatus, Boekelovia sp., Chaetoceros gracilis, Chaetoceros SS-14, Cyclotella sp., Nannochloropsis (Nanno-Q) sp. and Platymonas sp. A protocol for culturing and quantifying growth as doublings/day was established using culture tubes on shakers. Culture tubes served as spectrophotometric cuvettes and were read every 24 hours over a 5 day period. Nitrogen as NO_3, NH_4 and urea were tested for preference and optimal concentrations. Phosphate-P, Si (for diatoms) and the trace metal Fe were also tested for optimal concentrations. The vitamins B_{12} and thiamine were found to be significantly important in the growth of three of the species. Nitrate was observed to be the best nitrogen source in three of the seven algae tested. Ammonia and urea-N were shown to be significantly preferred nitrogen sources for two species. Iron as $FeNH_4$-citrate significantly increased the growth of two of three species tested as compared to $FeCl_3$ additions. This information will hopefully be helpful to the SERI Aquatic Species Program as well as others as a guide toward optimizing growth prior to the shift toward lipid production.

ENVIRONMENTAL CONTROL OF LIPID PRODUCTION IN DIATOM CULTURES

W. Barclay, P. Chelf, N. Nagle
Solar Energy Research Institute
Golden, CO

Nutritional stress (either nitrogen or silica) has been shown to be an effective inducer of algal lipid production. Exploitation of this induction effect could be vital in our effort to reach program goals of 15% photosynthetic efficiency and 50% of dry weight as lipid. To this end, an understanding of how nutrient stress increases lipid production and how environmental factors affect this induction is essential. Some environmental factors with the potential to affect lipid production under nutrient stress are inorganic carbon supply (CO_2, bicarbonate), pH, light intensity, and temperature. Preliminary experiments suggested that increasing bicarbonate concentration resulted in higher lipid yields under both nitrogen and silica stresses in Navicula sp. The preliminary experiments designed to examine this effect yielded interesting results but have not unequivocally answered the question of whether a bicarbonate effect exists. Based on this initial data, alternative experimental approaches are proposed to approach this question.

THE EFFECTS OF LIGHT INTENSITY ON LIPID PRODUCTION

N. Nagle and B. Barclay
Solar Energy Research Institute

Several conflicting reports have appeared in the literature, and in Aquatic Species Program research, on the effects of light intensity on lipid yield in microalgae. Some reports have suggested that high light intensity enhances lipid production while others have suggested that low light intensity improves lipid yield. We developed a simple procedure to evaluate light intensity effects on lipid induction in microalgae. A rotary screening apparatus was modified with neutral density filters to provide a variety of light intensities, from 100-1000 uE m^{-2} sec^{-1}. Additionally, a simple fluorometric procedure, utilizing nile red, was developed to quantify lipid yield. The data from several experiments suggest that higher light intensities, during stress induced lipid production, results in higher lipid yields in microalgae.

FLOW CYTOMETRY TECHNIQUES FOR SPECIES IMPROVEMENT*

Jean A. Solomon
Environmental Sciences Division
Oak Ridge National Laboratory

Lipid accumulation in three species of microalgae was investigated using flow cytometry (FCM) and transmission electron microscopy (TEM). Previous studies using batch cultures of algae have led to the assumption that lipid accumulation in microalgae is a gradual process requiring at least several days for completion. However, FCM reveals, through changes in the chlorophyll:lipid ratio, that the short time span required for individual cells to change metabolic state is short. Simultaneous FCM measurements of chlorophyll and nile red (neutral lipid) fluorescence in individual cells of nitrogen-deficient Isochrysis populations revealed a bimodal population distribution as one stage in the lipid accumulation process. The fact that two discrete populations exist, with few cells in an intermediate stage, suggests rapid response to a lipid trigger. Interpretations of light and electron microscopic observations are consistent with this hypothesis. The time required for an entire population to achieve maximum lipid content is considerably longer than that required for a single cell, due to the variation in response time among cells. In this study, high lipid cultures were sometimes obtained by using FCM to separate high lipid cells from the remainder of the population. FCM holds much promise for strain enhancement but considerable developmental work, directed at providing more consistent results, remains to be done.

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Biochemical Elucidation of Neutral Lipid Synthesis in Microalgae. J. B. Guckert and K. E. Cooksey. Department of Microbiology, Montana State University, Bozeman, Montana, 59717.

The mechanisms controlling lipid synthesis must be understood if one is to attempt their control and manipulation! The possibility of selecting high lipid producing organisms by genetic alteration bear little chance of success if the gene products (enzymes) specified as a result of genetic manipulations are unknown. We are therefore concentrating on the study of the regulation of lipid synthesis in microalgae in this project.
The pathways of lipid synthesis in all organisms examined so far are similar, but not identical. There are differences in the enzymes carrying out the reactions, but the reactions themselves do not vary much. In general, all pathways involve the conversion of acetate to its Coenzyme A derivative condensation with bicarbonate and subsequent addition of C-2 units to form a 16 carbon fatty acid. Further chain lengthening involves acetyl CoA or Malonyl CoA. The point to stress is that the synthesis of all lipids involves 2 or 3 carbon fragments derived from acetate in its coenzyme A form. There are variations as to how acetyl CoA is synthesized and there has been considerable controversy as to where this process takes place. Our research will focus on the pathway of synthesis of acetyl CoA and the further regulation of its metabolism. Acetyl CoA is at a branch point in metabolism, i.e., it can either be oxidized to 2 CO_2 and the CoA regenerated (TCA cycle) or it can condense with bicarbonate to form malonyl CoA and be committed to lipid synthesis. In some algae, nitrogen limitation fosters the second route. In some diatoms, silicon limitation does the same thing, i.e., the inability of a cell to divide induces increased lipid synthesis to take place. We believe that feedback control on citrate synthase, the consumer of acetyl CoA for the TCA cycle by -oxoglutarate or ATP shuts off the cycle and acetyl CoA is converted to a lipid 'sink'. How this control system can operate will be discussed.

This project started in September, 1986. Two organisms will be studied initially; both are high lipid producers. They are a Chlorophyte, Chlorella sp. and a member of the Bacillariophyceae, Cyclotella. The Chlorella responds to nitrogen limitation, by synthesizing increased lipid whereas the diatom responds to Si limitation in the same way.

Biochemistry of Neutral Lipid Synthesis in Microalgae

Lloyd Coleman, Barry Rosen and Steven Schwartzbach
Dept. of Biological Sciences, University of Nebraska, Lincoln Nebraska 28588

The improvement of lipid yields in microalgae requires an understanding of the physiological and biochemical basis for the partitioning of photosynthetically fixed CO_2 into lipids. Elucidation of the biochemical basis for enhanced lipid synthesis requires a reproducible method for inducing lipid synthesis. Since the most commonly utilized lipid trigger, nitrogen deficiency, rapidly reduces photosynthetic capacity, it is useful to separate effects of lipid triggers on photosynthetic efficiency from their effects on carbon partitioning. Initial studies have therefore used Euglena, a microalgae which grows equally well under photosynthetic and heterotrophic conditions as a model system. Based on the results obtained with Euglena, attempts were made to induce lipid synthesis in Chlorella SOl and Nannochloropsis salina.

Nitrogen deficient Euglena utilize exogenous carbon for the net synthesis of both carbohydrates and lipids in the light and the dark. Cells grown in the light accumulate less carbohydrate and lipid than cells grown in the dark. The lipid content of nitrogen deficient cells is two to fivefold higher than that of nitrogen sufficient cells. Nitrogen deficient and sufficient cells show little difference in the percentage of dry weight which is lipid. Growth at 33 C rather than 26 C increases the cellular lipid content. The higher growth temperature however inhibits the synthesis of lipid by nitrogen deficient cells. Anaerobiosis triggers lipid synthesis in both nitrogen sufficient and deficient Euglena. In N sufficient cells, the degradation of storage carbohydrates provides carbon for lipid synthesis while in N deficient cells, endogenous carbon is not efficiently mobilized for lipid synthesis; lipid is only synthesized when a source of exogenous carbon is available.

Chlorella SOl grows well in the dark with glucose as the sole source of carbon and energy. The provision of additional glucose to nitrogen deficient cultures grown in the light on glucose failed to stimulate lipid synthesis. Contrary to the results with Euglena, the exogenous carbon was used almost exclusively for the synthesis of carbohydrates. When Chlorella SOl was grown photosynthetically to nitrogen deficiency, cellular lipid content increased. The partitioning of carbon by Chlorella SOl is dependent on the source of carbon.

We have been unable to maintain axenic cultures of Nannochloropsis salina. When grown photosynthetically, the cell number, lipid per cell and % lipid on a dry weight basis was a function of the initial initial NH_4Cl concentration of the medium. As cells entered nitrogen deficiency, cellular lipid content and dry weight increased while chlorophyll decreased. The lipid content of nitrogen deficient cells was at least twice that of nitrogen sufficient cells. The addition of nitrogen to nitrogen deficient cultures stimulated cell division and chlorophyll synthesis. Lipid per cell decreased upon nitrogen supplementation clearly indicating that the increased lipid content was due to nitrogen deficiency.

Taken together, the results with Euglena and Nannochloropsis salina indicate that lipid accumulation in these algae can be reproducibly triggered by nitrogen deficiency. Under photosynthetic conditions, the extent of lipid synthesis may be limited not by the levels of lipid synthesizing enzymes but rather by the availability of carbon. The development of reproducible methods for the induction of lipid synthesis in Euglena and Nannochloropsis salina will enable us to correlate changes in lipid levels with the activity of lipid synthesizing enzymes.

BIOCHEMICAL ASPECTS OF LIPID ACCUMULATION IN SILICON-DEFICIENT DIATOMS

Paul Roessler
Biotechnology Branch
Solar Energy Research Institute
Golden, Colorado 80401

Although many algal species have been observed to accumulate lipids in response to nutrient deficiency, the biochemistry of this process is unknown. This type of information will be essential, however, before algae with increased lipid production rates can be obtained via genetic engineering or biochemical modification. The following research was therefore initiated in an effort to identify some of the factors responsible for lipid accumulation in nutrient-starved algae.

Three different diatom species were examined with respect to lipid accumulation under silicon-deficient growth conditions. The lipid contents of Cylindrotheca fusiformis and Cyclotella cryptica cells increased significantly as a result of Si-deficiency, but this was not observed with Thalassiosira pseudonana. However, the neutral lipid content of all three species increased by at least two-fold under conditions of Si-limitation. Additional experiments indicated that the partitioning of newly assimilated carbon was rapidly and significantly altered in response to Si-deficiency in C. cryptica. The percentage of carbon partitioned into chrysolaminarin was reduced by 50%, while the fraction partitioned into lipid was nearly doubled. This was apparently due to differential amounts of decrease in the absolute rates of carbon assimilation into these two compounds. Further experiments with C. cryptica indicated that Si-deficiency also induced a slow redistribution of previously-fixed carbon from non-lipid materials into lipids, increasing the initial lipid mass by 32% during the first 12 h of Si-deficiency. There also appeared to be a slow conversion of polar lipids into triacylglycerols in Si-starved cells.

In order to better understand the biochemical basis of these changes in carbon partitioning, we have begun to characterize some of the key enzymes of carbon metabolism in diatoms. The first phase of this research, designed to elucidate the pathway of chrysolaminarin biosynthesis in C. cryptica, indicated that UDP-glucose serves as the glucosyl donor for the synthesis of this carbohydrate. When UDP-[^{14}C]glucose was added to cell-free extracts, a ^{14}C-labeled glucan was produced which had a median molecular weight of 4600 (based on gel filtration chromatography) and could be hydrolyzed by Penicillium laminarinase (a beta-1,3-glucanohydrolase). Partial acid hydrolysis of the glucan resulted in the production of glucose and laminaribiose, but not cellobiose, providing additional evidence that the glucan was chrysolaminarin. UDP-glucose was formed in cell-free extracts of C. cryptica via the action of UDP-glucose pyrophosphorylase. When assayed in the direction of UDP-glucose formation, this enzyme had maximal activity at pH 7.8 and was greatly stimulated by Mg^{2+} and Mn^{2+} ions. 3-phosphoglycerate and inorganic phosphate had little effect on enzymatic activity, and the enzyme was relatively insensitive to feedback inhibition from UDP-glucose (K_i>1 millimolar). The K_m constants for glucose-1-phosphate and UTP were determined to be 41 and 56 micromolar, respectively.

Future experiments will investigate the effects of Si-deficiency on the activity of UDP-glucose pyrophosphorylase and various lipid biosynthetic enzymes.

EFFECTS OF INDUCTION STRATEGIES ON THE GROWTH RESPONSE OF DIATOMS, ON CHAETOCEROS (SS-14), CYCLOTELLA DI-35 AND HANTZSCHIA DI-60 WITH EMPHASIS ON LIPIDS

Shobha Sriharan* and Davinderjit Bagga**
*Division of Natural and Applied Sciences
Selma University, Selma, Alabama 36701

**Biology Department
Miles College, Birmingham, Ala. 35208

Abstract

The objectives of the research reported here were to determine the effects of induction strategies (nitrogen and silica variations and variable temperatures) on lipid production in diatoms, chaeteceros (SS-14), Cyclotella DI-35 and Hantzschia DI-60. The diatoms were batch cultured in Nitrogen (N) and Silica (S) concentrations as N-sufficient (NS) (600/uM) and N-deficient (ND) (300 /uM); S-sufficient (SS) (1mM) and S-deficient (SD) (250) /uM) media at two different temperatures (20°C and 30°C) under continuous illumination. The biomass was harvested during the log phase and during the stationary phase in NS, SS and ND, SD treatments respectively. The ash-free dry mass (AFD) was measured. Total lipids were extracted from harvested cells by extracting twice with methanol followed by two additional extractions with chloroform-methanol (1:1 v/v). The crude lipids were separated into neutral lipids and polar lipids by column chromatography on silicic acid column. The fatty acid of chaeteceros (ss-14) was analyzed by gas liquid chromatography by saponifying algal cells followed by methylation and extraction of methyl esters.

The results showed that there was a significant increase in cell density and ash-free dry mass (AFD) when these three diatoms were grown in NS and SS conditions as compared to ND and SD conditions at 30°C as well as 20°C. At 30°C, the growth response was higher than at 20°C. The total lipids (% of AFD) were higher in ND and SD conditions than in NS and SS conditions at both 30°C and 20°C in each of these three diatoms. The amounts of neutral lipids and polar lipids were also greater when these diatoms were grown in ND and SD media than in NS and SS media at 30°C and 20°C. The ratio of neutral lipids was higher than polar lipids in each case under all experimental conditions.

The results suggest that maximum AFD production was attained when the cultures reached maximal cell density. The lipid composition, total lipids, neutral lipids and polar lipids were higher in nitrogen and silica stressed conditions. The observations were similar at 30°C and 20°C, but the rate of lipid production was higher at 30°C in all the three diatoms tested.

The effect of silica concentration (S-deficient) was significant on the production of fatty acids 14:0, 16:1, 16:0 and 18:1 in Chaetoceros (SS-14). The production of these fatty acids was higher at 30°C than at 20°C.

EFFECTS OF FLUCTUATING ENVIRONMENTS ON THE SELECTION OF HIGH YIELDING MICROALGAE

John R. Benemann and David M. Tillett
School of Applied Biology, Georgia Institute of Technology

The mass culture of microalgae for the production of lipids requires detailed understanding of both the physiology of lipid biosynthesis by the selected algal strain and of the factors making this strain competitive in mass culture systems. Mass culture systems will, by their nature, exhibit fluctuations in key parameters affecting algal growth, productivity, and competitiveness: temperature, pH, pO2, pCO2, light, and nutrient availability. Both controllable (pond operations) and uncontrolable (climatic) factors will affect the fluctuations in these parameters. Our project has for its overall objective to quantitate the effects of such fluctuations on lipid biosynthesis and species competition.

Using "Nanno Q II" as a test organism, because of the relatively high lipid content achievable in laboratory cultures, we have followed lipid productivity and overall photosynthetic efficiencies in both batch and continuous (cyclostat) cultures as a function of light and nitrogen supply. We found that in batch cultures biomass and lipid productivity will be maximized regardless of cell density if the culture was light limited before the nitrogen limitation set in. This relationship held up to about 1.4g/L dry weight (the highest tested), at which point productivity was still near maximal. We conclude that in mass cultures it should be possible to operate the ponds at as high a cell density as feasible with high productivity, before switching to N limitation. When cultures were shifted to a higher light input, after being subjected to N limitation, lipid productivity during N limitation actually increased as compared to the control culture grown at the higher light input throughout the experiment. This suggests that in a two stage growth system the first, N sufficient, stage can be relatively smaller in size than predicted from constant light environment data. Finally we compared batch to continuous cultures and found that lipid productivities were substantially less, across the board, for continuous (cyclostat) cultures in both N sufficient and N deficient experiments.

We have carried out a number of species competition experiments with several of the algae used in the SERI program (*Chlorella*, *Cyclotella*, *Chaetoceros*, etc.). We found that periodic high concentrations of O2 influenced the outcome (species dominance) of the experiment most significantly, with temperature fluctuations being less important and fluctuating pH having no noticeable effect. Results from an experiment in which dilution rate was varied suggested that there is a threshold growth rate above which the transients in start- up conditions influence the outcome of the experiments. These studies are at a preliminary stage and the conclusions can not yet be extrapolated beyond the species and conditions tested.

To better characterize the actual environmental fluctuations that will be experienced by algal cultures in outdoor systems we have developed a computer model which integrates site specific climatic data with pond design and operating inputs to predict the variations in temperature, pH, and pO2. We will present the model and its application to guiding future research in this area.

Temperature Effects on Microalgal Photosynthetic Efficiency

R. Radmer and P. Behrens
Martek Corporation

ABSTRACT

Algal cultures grown in an outdoor production facility will experience daily and seasonal fluctuations in temperature, and thus these cultures will exist under less than ideal temperature conditions. As a result, it is important to determine the effect of changes in temperature on biomass productivity, since this information will be valuable in predicting the potential yield of algae grown in large-scale outddor culture. The objectives of this project were to: (1) determine the maximum growth rate of selected algae under conditions in which neither nutrients, carbon dioxide, nor light were limiting, and (2) determine the effect of changes in temperature on biomass productivity and photosynthetic light efficiency.

The maximum growth rates of Ankistrodesmus, Chaetoceros, Chlorella, Cyclotella, Navicula, Scenedesmus, and Skeletonema were determined to be 2.6, 4.6, 4.9, 3.9, 2.0, 3.3, and 2.0 doublings per day, respectively. The relationship of biomass productivity to cell concentration for a Scenedesmus continuous culture maintained at three different temperatures (18, 25, 32 °C) and high light intensity (295-361 $\mu E/m^2/sec$) was determined. Biomass productivity was maximal at 25 °C, and the relationship of productivity to cell concentration was temperature dependent (i.e. the cell concentration required to obtain maximum productivity changed significantly as a function of temperature).

GENETIC VARIATION IN OIL-PRODUCING MICROALGAE

Jane C. Gallagher
Biology Dept
City College of N.Y.
New York, N.Y. 10031

Strains of Amphora coffaeformis, Nannochloropsis spp., and Nannochloris spp. were examined for genetic differences by gel electrophoresis and photoadaptation characteristics. Genetic differences were found among strains within each genus.

No two clones of Amphora coffaeformis obtained from established culture collections were determined to be genetically identical. Newly isolated strains of this organism obtained from Woods Hole from a series of sites within 300m of each other showed greater genetic diversity than planktonic diatoms examined previously. There was no clear relationship between environmental factors and the frequency of different genotypes. Scanning electron microscopy of the clones revealed morphological variation and clones with similar frustule morphologies were found to be more closely related to each other than those with different morphologies. The presence of a relatively high proportion of heterozygotes and the lack of linkage groups among alleles at different loci indicate that interbreeding among gentoypes may be occurring in natural populations of Amphora.

No two clones of Nannochloropsis spp. obtained from the Culture Collection of Marine Algae were determined to be genetically identical although they had identical morphologies. The wide variety of electromorphs represented in these collections and the high variance in growth rates, cell composition and photosynthetic rates indicate that these strains are probably not very closely related and that the entire group requires taxonomic revision.

Clones of Nannochloris spp. were all isolated by Lewin and Chang from the Carribean. These clones showed genetic differences, but were more similar to each other than were the clones of Nannochloropsis spp. This may be due to different sampling strategies used to isolate new strains. As a group, the strains of Nannochloris spp. tended to have lower growth rates, chlorophyll a per cell and photosynthetic rates in high light than did Nannochloropsis spp.

These patterns of genetic variation can be used to identify groups of organisms most promising for future genetic manipulation.

KELP GENETICS

M. Neushul
Neushul Mariculture Incorporated/U.C.S.B.

Work on giant kelp biomass production began at NMI in June, 1980 when we sub-contracted with General Electric Company and subsequently with Seri to install, plant, harvest, measure and analyze the results of an in-the-sea macroalgal farm. We were able to show that the giant kelp was equivalent to sugar-cane in it's productivity under cultivation (Neushul and Harger, 1985). The importance of genetic strain selection soon became apparent and a seedstock collection of pedigreed lines was established. We were able to make both intergeneric and interspecific hybrids of pacific coast kelps (Neushul, 1982; Lewis, Harger and Neushul, 1986). Gametophytes from hgh-yielding kelps, obtained either from a pedigree or mass-selection approach, can be held for long periods of time without loosing their fertility.

The NMI contributions to the development of algal biomass technology in general (Barclay and McIntosh, 1986) is modest indeed when viewed historically. It is a little known fact that the kelp beds of Califoria provided the basis for a million dollar cordite and potash industry when Germany cut off its supply of potash to the United States during the first world war. Today, the most active centers for algal biomass production are China, Korea and Japan. In China in particular, the importance of genetic selection was clearly demonstrated by the late T. C. Fang who developed new strains of Laminaria that grow well in warm water and have increased iodine content.

Current research at NMI focuses on the development of new techniques to genetically manipulate kelps. Progress has been made in bacteria-free culture, the control of gametogenesis, the measurment of ploidy levels, tetrad analysis and the use of mutagens. Since kelp gametophytes are long-lived, have no somoclonal variation, they can be used as a "seedbank" of genetic types. It seems logical to screen them under laboratory conditions, and retain those that yield kelp plants that produce specific products, or are particularly productive. We have found that there is variability in the growth rates of gametophytes, but have not yet been able to link high gametophytic growth with high sporophytic growth.

The problem of "genetically domesticating" macroalgae can be extended to other algal groups, where cultivation methods, DNA measurement methods, mutagenesis, transgenosis, and many other techniques are yet to be tried. The great advantages of exploiting the genetic diversity of the algae are not always evident. However we have found that some algae produce products that could be very valuable. Genetically domesticating such plants will certainly be of value to the SERI Aquatic Species Program.

ABSTRACT

CHARACTERIZATION OF VIRUSES INFECTING CHLORELLA-LIKE ALGA

RUSSEL H. MEINTS (1), ANNE M. SCHUSTER (1), AND JAMES L. VAN ETTEN (3). (1) SCHOOL OF BIOLOGICAL SCIENCES AND (3) DEPARTMENT OF PLANT PATHOLOGY, UNIVERSITY OF NEBRASKA

Exsymbiotic, Chlorella-like, eukaryotic green algae act as hosts for a group of DNA containing viruses. We have isolated over 100 plaque forming viruses from fresh water collected from ponds, streams and rivers throughout the United States. For this study 15 representative viruses from a single Nebraska water sample are being subjected to detailed analysis. Based on previous studies, we have described a prototype virus, PBCV-1, a large, dsDNA (330 kbp) virus which infects three exsymbiotic strains of Chlorella. PBCV-1 is easily assayed since it will form plaques on lawns of the alga. Viral attachment and infection is rapid and and the entire lytic cycle of the virus is completed within 8 hrs. This is the only example of a plant- virus interaction which lends itself to manipulation using the technology established for study of bacteriophage.

When the viruses ultimately lyse their algal hosts, the resulting lysate is a rich source of cell wall degrading enzyme(s), which we have called lysin. Treatment of cells with lysin results in the complete degradation of the algal wall, leading to the formation of protoplasts. Our proposal, for which studies are now underway proposes to use these protoplasts as useful partners in creation of somatic hybrids and as hosts for direct transformation with autonomously replicating vectors created from all or portions of the viral genomes. In general, conventional means for improvement of plant species revolve about the transfer, through conventional sexual hybridization, of desired genetic trains. For many microalgae, for which sexual mating systems have not been demonstrated, alternative methods such as those described here, for transfer of genetic traits are required.