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Screening and Characterizing Oleaginous Microalgal Species from the Southeastern United States

A Final Subcontract Report

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Normal, Alabama

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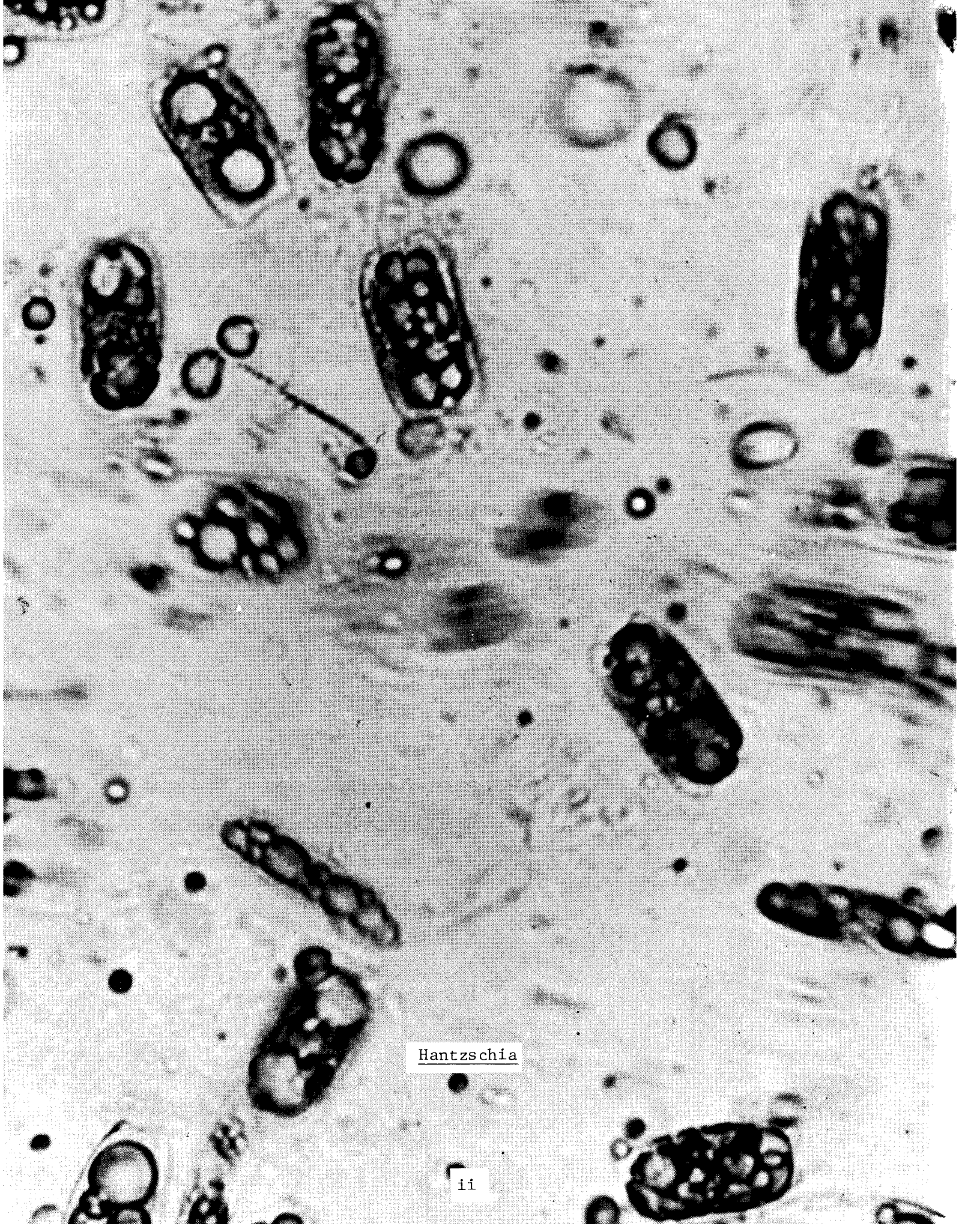

Hantzschia

FOREWORD

This report is the final report for FY 1984. The work was performed under subcontract to SERI with funds provided by the Biomass Energy Technology Division of the U.S. Department of Energy and the Historically Black College and University Program.

Robins P. McIntosh
Aquatic Species Program

Approved for the

SOLAR ENERGY RESEARCH INSTITUTE

Stanley R. Bull, Acting Director
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SUMMARY

Objectives

The objectives of this work were to (1) collect microalgae from freshwater and saline habitats in the southeastern United States; (2) isolate and define culture conditions of high lipid-yielding strains of microalgae, and (3) determine the lipid yield and growth rate from selected strains of microalgae.

Discussion

The work under this subcontract emphasized collecting, isolating, and characterizing microalgae from habitats of the southeastern United States. Collections were made from habitats that are exposed to extreme environmental conditions, such as intertidal salt marshes and temporary ponds, puddles, and marshes. Microalgae were isolated and screened for tolerance to high temperature and light intensity and for media requirements. Those species that are capable of growth in outdoor light and high temperatures were then grown in culture and qualitatively evaluated for lipid yield. The species that are designated "most promising" were finally grown under various culture conditions that generally result in lipid accumulation and will be analyzed for total lipids, protein, and carbohydrates.

Conclusions

Collection trips were made to Dauphin Island near the Alabama coast during the summer of 1984. Over sixty strains were isolated, and of these six were ranked as good growers. Two diatoms were isolated that are of particular interest because of their ability to accumulate high lipids. Cyclotella tolerates high temperatures (30°-35°C), grows at moderate salinities (15-25 parts per thousand), and with nitrogen stress accumulates 42% of its dry weight as lipid. Hantzschia is a large diatom that also grows well at elevated temperatures and full strength seawater. Hantzschia can accumulate as much as 66% of its dry weight as lipid.

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ABSTRACT

The purpose of this study was to select and characterize promising algal species which tolerate high light intensities, temperature variations and accumulate lipids. Samples have been collected from freshwater and saltwater locations in the State of Alabama and intertidal regions of the Gulf of Mexico. Samples were screened through a multi-step process. Selected species: Cyclotella, Nitzschia, Chlorella, Scenedesmus and Ankistrodesmus, have been examined for growth requirements. Approximate cellular composition of these species was determined.

INTRODUCTION

In recent years, interest in microbial lipids has been renewed because of an urgent need for utilization of alternative renewable resources as carbon sources for production of hydrocarbons and lipids. Since 1980, SERI/DOE has been interested in microalgal liquid fuels (McIntosh, 1984).

Microalgal species are capable of producing biomass yields containing high percentages of oils (Aaronson, et. al., 1980). Microalgal systems can use low value natural resources, such as saline water and arid lands, therefore offering the potential for large biomass energy contributions without competition for prime agricultural or forest land. The growth of microalgal biomass and lipid harvesting represent potential for harvesting solar energy products. The microalgal energy technology offers an attractive long-term renewable energy option, with the following advantages:

° Algal lipids are in general high in polyunsaturated fatty acids (Miller, 1962), and are comparable to that of plants: linseed, cotton-seed oils (Nurris, 1983).

° Microalgae have the ability to survive in extreme environments.

° Cells of many algal species have a negligible ash content.

One of the methods by which the energy storage capacity of photosynthesis can be maximized is by controlling the metabolism of the organism. Tuning the metabolism of algae can lead to enhanced production of energy-rich compounds such as fatty acids and glycerol. A single algal species may show remarkable variation in its metabolism, according to the conditions to which it is exposed, such as carbon dioxide supply, light intensity, temperature, nutrient concentrations, and salinity (Holm-Hansen, et. al., 1959). Changes in the supply or consumption of metabolitis may have

considerable effects on metabolic patterns. The accumulation of energy storage compounds in algae, such as fats and oils, can be induced by manipulating the environmental conditions under which the algae are grown (Shifrin and Chisholm, 1981).

Nutrient deficiencies generally lead to a decrease in protein and photosynthetic pigments and an increase in energy-rich products such as carbohydrates and lipids (Healey, 1973). Nitrogen starvation in particular can lead to remarkable changes in algal cell composition (Fogg, 1959). Opute (1974) demonstrated that lipids accumulate in the diatom Nitzschia palea, under N-deficient conditions. Cyclotella cryptica produces high lipids in N-deficient media (Werner, 1966). Diatoms (Bacillariophyceae), mostly in their stationary growth phase, accumulate fats. Chlorella pyrenoidosa (Chlorophyceae), produces high concentrations of lipids, from 28% to 70% dry weight (Fogg, 1959) when grown in N-starved cultures. The freshwater alga Botryococcus braunii (Chlorophyceae), accumulates up to 75% of its total dry weight in hydrocarbons when subjected to nutritional deficiencies (Maxwell, et. al., 1968). The resting stage of this alga yields a distillate which is particularly rich in quality fuels: 67% petrol, 15% turbine, and 15% diesel (Wake and Hillen, 1980). Even during silicon starvation, the marine diatom, Navicula pelliculosa, accumulates lipids (Coombs, et. al., 1967). With aging, nutrients become exhausted; this is then reflected in lipid content increase and changes of fatty acid composition (e.g., Euglena gracilis, (Gomez, et. al., 1974), and Nitzschia, (Opute, 1974; Badour and Tadros, 1965)).

Fat production is also stimulated by light. Spoehr and Milner (1948), showed that N-deficient Chlorella pyrenoidosa attains a greater lipid content at high light intensity than at low light intensity. The length of light period as well affects the content and composition of fatty acids of Nitzschia palea (Opute, 1974) and Nitzschia closterium (Orcutt and Patterson, 1974). Ochromonas danica (Chlorophyceae) accumulated lipids up to 53% dry weight when cultured at high temperature (Aaronson, 1973). The fatty acid composition of the diatom Coscinodiscus eccentricus (Pugh, 1971) showed variations in different salinity media. Other halophilic species such as Dunaliella spp. (Chlorophyceae), when subjected to high salinity media, modify their intercellular environment by accumulating compatible solutes such as glycerol, which can reach 85% dry weight of the alga (Ben-Amotz and Avron, 1973). In a better characterized system, the diatom Phaeodactylum tricornutum growing in sea water produced high yields of lipids (Raymond, 1981).

From previous reports it is evident that hydrocarbon production by algae is occurring in natural systems and can be maximized by the application of a variety of environmental conditions (Shifrin and Chisholm, 1981). In order to select promising algal species as potential producers of oils for energy technology, these growth conditions need to be identified.

In this project, the most promising lipid forming species collected in the southeastern U. S. were screened and characterized with respect to their growth properties and oil-liquid productivity.

Objectives

The long-range goal of this project is to establish an adequate biological resource pool of oil-producing microalgal species from the southeastern United States. Most of the oleaginous algal species at SERI have been isolated from the southwestern United States, while southeastern United region has not yet been approached.

The specific objectives of the research reported herein were:

° To collect algal samples from freshwater and saltwater resources in the State of Alabama.

° To isolate oleaginous algal species capable of growth under high temperature and light intensity.

° To characterize oleaginous species, for their temperature, salinity and light tolerances.

° To determine the nitrogen source requirements for the selected species.

° To quantify the lipid accumulated by the selected strains, under nitrogen deficienty and nitrogen sufficiency.

Significance

The specific research objectives stated above are fundamental to the acquisition of the following benefits and goals:

° Isolation of oleaginous algal species to be included in SERI algal culture collection, to be used as an algal bank for researchers and the private sector.

° Defining the conditions which increase lipid production in oleaginous microalgae, e.g., temperature, light, salinity, and nutrients.

° Applying laboratory results for outdoor mass production and technology.

° Production of high energy compounds.

This report describes what has been accomplished in the period February 1984 - January 1985.

MATERIALS AND METHODS

Sites of Sampling

The northern region of the State of Alabama is characterized by fresh-water resources, e.g., lakes, rivers and ponds. The soil has a high percentage of carbonate rocks, while that of the southern region has sand and gravel. The temperature ranges from 2-18°C in January and 21-35°C in July. The Gulf Coast of Alabama contains an exceptional variety of representative conditions. The salt waters of the Gulf of Mexico are mixed with fresh inland waters to form all grades of brackish water, from fresh to almost as salty as the Gulf. In these waters exist a wide variety of living organisms that can tolerate a wide range of salinities. The intertidal region and Dauphin Island represents fishing sites, rich in algal species.

Field Trips and Sample Collection

Field trips have been conducted to: rivers, lakes, ponds, streams, and swamps under environmental stress (Fig. 1). Fresh water and marine algal samples were collected from dry and wet inhabitants. Temperature, pH, and salinity of samples were recorded. Salinity was measured with a salinometer or a conductance meter. Collected samples were enriched with nitrate and phosphate media to maintain the dominant species. They were protected from temperature changes during the trips, by being kept in a cooler. Alabama A. and M. University is about 500 miles from the Gulf Coast. Therefore it was difficult to bring sea water from the Gulf to use for enrichment growth media of algal species. Private cars have been used for the trips over the weekend or for longer periods, depending on the distance.

Algal Sample Processing

Screening Procedure: A screening process has been designed for this objective. It involves many sequential steps for selection of oleaginous algal species tolerant of high temperature and light intensity (See Fig. 2).

Growth Conditions

Culture Room: A small room (3m D x 2.45m W x 2.14m H), was designed specifically for this project. It has been provided with shelves, which have been illuminated with cool white light fluorescent tubes. Light intensity varied from 400 to 500 foot-candles on the shelves. Intermittent illumination was used for culturing (14h:10h light dark cycle).

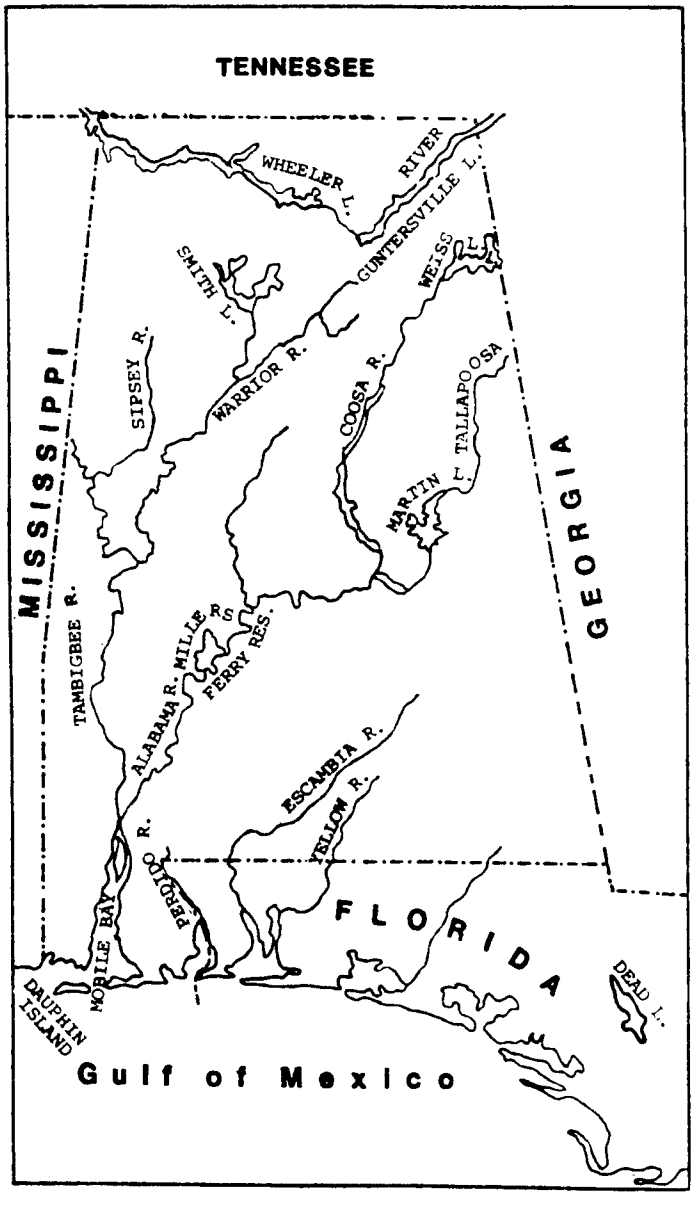

Figure 1. Locations of Sampling Sites in Alabama

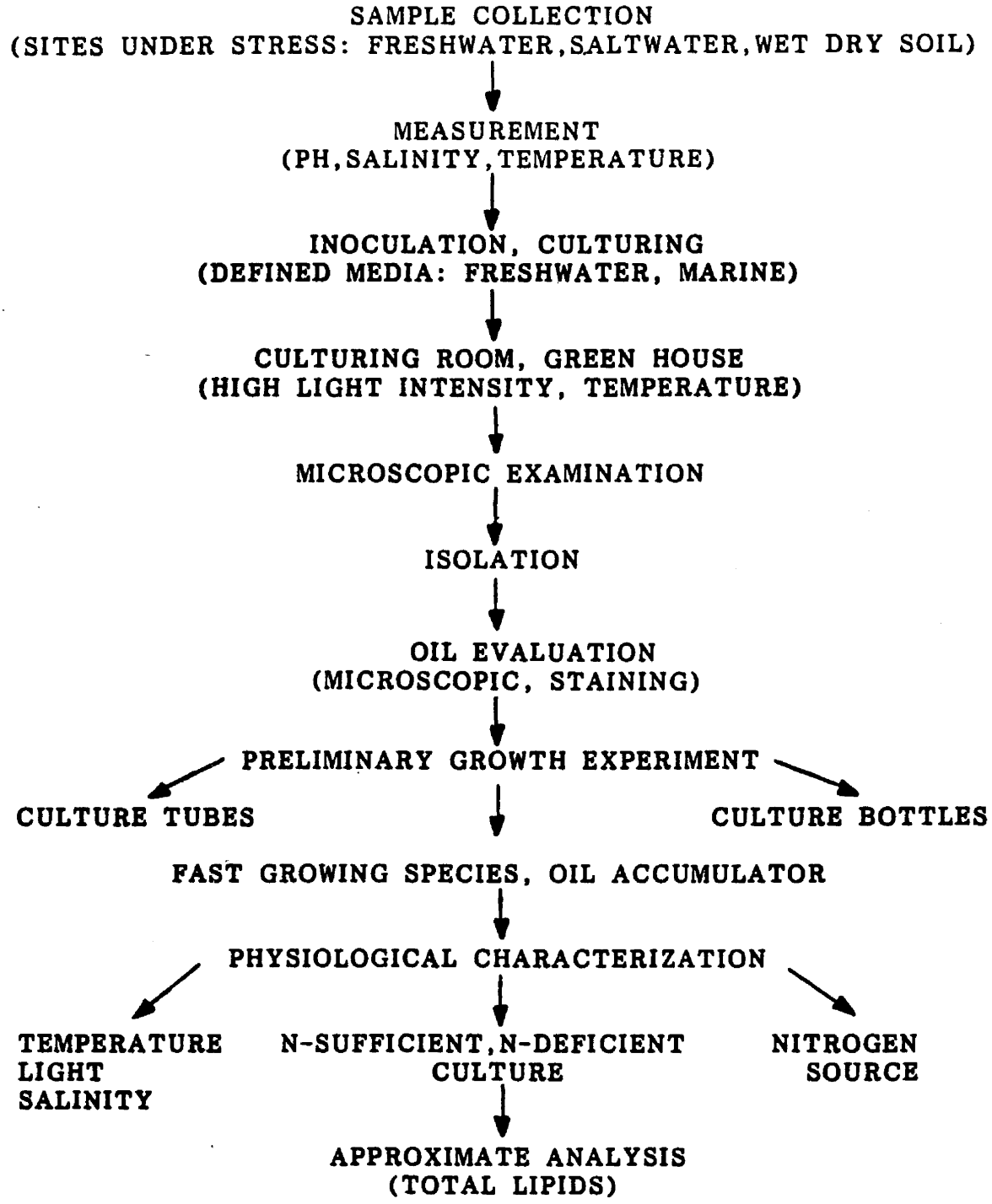

Figure 2. Screening Procedure of Oleaginous Algal Species

Growth Media

Growth media for fresh water and marine algal species were used for isolation. Defined media were supplemented with artificial sea salts "Instant Ocean" (Aquarium System, Inc., East Lake, Ohio), at different strengths.

Among the principal basic media are:

° Bold Basal, pH 6.6 (Nichols and Bold, 1965): $NaNO_3$ 0.25g: $CaCl_2$ 0.25g; $MgSO_4$ $.7H_2O$ 0.75g; K_2HPO_4 0.075g; KH_2PO_4 0.175g: $NaCl_2$ 0.025g, in 1 L of distilled water.

° Chu no 10, pH 6.5 - 7.0 (Chu, 1942): $Ca(NO_3)_2$ 0.04g; K_2HPO_4 0.01g; $MgSO_4$ $.7H_2O$ 0.025g; Na_2CO_3 0.02g; Na_2SiO_3 0.025g, in 1 L of distilled water.

° "f/2", pH 7.5 (Guillard and Ryther, 1962): $NaNO_3$ 0.075g; NaH_2PO_4 $.H_2O$ 0.005g ; $NaSiO_3$ $.9H_2O$ 0.030g; Thiamin. HCl 0.1mg; Biotin 0.5 ug; B_{12} 0.5 ug, in 1 L of sea water, vitamins have been omitted from the medium except in some species.

To all the above mentioned media, trace elements, 1 ml was added. The mixture of trace elements (Kates and Volcani, 1966) consisted of: H_3BO_3 0.568g; $ZnCl_2$ 0.0624g; CuCl $.2H_2O$ 0.268g: Na_2MoO_4 $.2H_2O$ 0.252g; $CoCl_2$ $.6H_2O$ 0.42g; $FeSO_4$ 1.36g; $MnCl_2$ $.4H_2O$ 0.36g, in 1 L of distilled water.

Isolation

Algal species have been isolated by streaking enriched agar plates or by micropipetting under a light microscope. Species were chosen on the basis of rapidity of growth and on dominance.

Qualitative Evaluation of Lipids

Oil accumulation was identified by microscopic examination. For freshwater algal species, Lien's (1984) staining technique for lipids has been used.

Species Identification

Isolates were identified in our laboratory, others were identified by SERI.

Preliminary Cultures

Culture Test Tubes: A multi-wrist shaker was modified to simultaneously shake 150 culture test tubes. Isolates were inoculated in 3 ml growth media and shaken under 500 ft. C and 29-30°C. Growth was evaluated visually by the same person and scored.

Culture Bottles: Small culture bottles (60 ml capacity), containing 45 ml growth medium, have been inoculated with algal strains, and aerated, using a small air pump. Growth of the cultures was evaluated visually by the same person and scored.

Growth Measurement

Temperature, Salinity, Light: Selected species were evaluated for temperature, salinity and light tolerances on a gradient plate. Temperature could be adjusted in range from 10°C to 40°C. Illumination was provided by eight cool white fluorescent tubes (40 W) with a 14H:10h light, dark cycle. Different light intensities were obtained by varying the distance between the cultures and light source. Selected algal species were cultured in small bottles (60 ml capacity) containing 20 ml growth medium enriched with sea salt or sodium chloride. Triplicate culture bottles were inoculated from stock cultures in the exponential phase and were shaken once a day. The temperature and salinity combinations on the gradient plate were evaluated by determining cell concentrations when cultures reached the stationary phase.

Nitrogen Source: Selected algal strains were grown in culture flasks of 100 ml capacity containing 50 ml basic medium, and enriched with different concentrations of urea or nitrate. The treatments were run in triplicate and inoculated with exponentially dividing cells. The culturing flasks were placed on the shelves in the culture room and were shaken by hand twice a day. Yields of all treatments were measured by cell counting.

Proximate Analysis

Batch Culturing: Experimental amounts of algal cells were grown in one liter bottles, containing 800 ml sterile growth medium, by inoculating them with 50 ml of preadapted rapidly growing culture in a 125 ml erlenmeyer flask. The batch cultures were aerated with air mixed with 3% CO_2 at the rate of 150 ml per minute. Cultures were illuminated continuously by placing them in front of a bank of six cool white fluorescent lamps (32 W). Light intensity, measured at the surface of culture bottles with a light meter, was 600 foot candle. The cultures were grown in a water bath kept at 29-30°C by the use of a heater-thermostat combination.

Analytical Method: Samples of definite volumes, were withdrawn from the cultures, centrifuged, washed and suspended in isotonic salt solution (Spencer and Lien, 1984). Triplicate samples of the algal suspension were taken for each determination. The mean value of these triplicates was recorded. The following determinations were carried out:

° Total dry weight: The cells were dried for 6 hours at 95°C.

° Total carbohydrates: Carbohydrates determinations were made, using a phenol-sulfuric acid method for analysis of algae reported by Kochert (1978).

° Total proteins: Analysis of total proteins was conducted using a heated biuret - Folin procedure modified by Dorsey, et. al. (1978).

° Total lipids: Total lipids were determined gravimetrically as described by Spencer and Lien (1984).

° Calculations: The proximate chemical composition of algae was expressed as values for: total protein, total carbohydrates, total lipid, all expressed as percentage of cellular organic weight.

RESULTS AND DISCUSSIONS

Sample Collection and Preliminary Growth Experiments

Data from sample collections, field trips, and processing according to the methods, are summarized in table 1. Following the use of the schematic diagram (Fig. 2) for screening the samples for oleaginous algal strains, the growth of the isolates in preliminary experiments was evaluated visually and represented by signs (+) in Table 1. The preliminary growth experiments were designed to test the growth of the isolates in liquid media. Most of the isolated strains were unialgal or mixed species, but not axenic. No significant bacterial growth was observed during the growth experiments.

The following strains were selected for characterization on the basis of fast growth rate and microscopic identification of oil accumulation in the cells:

° Bacillariophyceae (Diatoms):
Cyclotella sp. DI-35
Nitzschia sp. TR-114

° Chlorophyceae (Unicellular Green):
Chlorella sp. MB-31
Ankistrodesmus sp. TR-87
Scenedesmus sp. TR-84

The diatoms and unicellular green species were cultured in f/2 (triple strength) and BBL media.

Table 1. Algal Species Isolated From Field Trips; Water Characteristics; Preliminary Growth and Evaluation

No.	Species	Date of Collection	Temp.	pH	Salinity ppt.	Growth Condition	Preliminary Growth, Eval.
AR[1]-47	Nitzschia sp.	October 1983	26°C	8.0	0	FW	+
AR-67	Nitzschia sp.	October 1983	26°C	8.0	0	FW	++
AR-68	Navicula sp.	October 1983	26°C	8.0	0	FW	++
MB-63a	Chlorella sp.	October 1983	28°C	7.2	0	FW	+
MB-63b	Flagellate	October 1983	28°C	7.2	0	FW	+
MF-7B	Motile (green)	November 1983	24°C	7.8	0	FW	++
MF-8	Motile (green)	November 1983	24°C	7.8	0	FW	++
DI-31	Diatoms, Chlorella	November 1983	23°C	7.6	12	SW	+
DI-35	Cyclotella	November 1983	22°C	7.5	15	SW	++++
DI-34	Navicula	November 1983	22°C	7.5	15	SW	+
DI-32	Achnanthes	November 1983	22°C	7.5	15	SW	+
DI-42	Melosira	November 1983	22°C	7.5	15	SW	++
DI-51	Melosira, Phaeodactylum	November 1983	23°C	7.2	20	SW	+
DI-31	Navicula, Phaeodactylum	November 1983	23°C	7.2	20	SW	+
DI-34	Navicula, Nitzschia	November 1983	24°C	7.5	18	SW	++
DI-38	Rhizosolenium	November 1983	24°C	7.5	18	SW	+
DI-38a	Phaeodactylum, Nitzschia, Cocconeis	November 1983	24°C	7.5	18	SW	+
BW-46	Scenedesmus	November 1983	24°C	7.5	0	FW	+++
BW-52	Scenedesmus, Ankistrodesmus	November 1983	22°C	7.5	0	FW	+++
SL-1	Flagellate (green)	November 1983	25°C	6.8	0	FW	+++
SL-2	Chamydomonas	November 1983	25°C	6.8	0	FW	+++

Table 1. Continued

No.	Species	Date of Collection	Temp.	pH	Salinity ppt.	Growth Condition	Preliminary Growth, Eval.
SL-3	Motile (green)	November 1983	25°C	6.8	0	FW	++
SR-2	Chlorella	November 1983	22°C	6.5	0	FW	+
SR-3	Chlorella	November 1983	22°C	6.5	0	FW	+
MB-31	Chlorella	June 1984	30°C	7.2	0	FW	++++
MB-81	Motile (green)	June 1984	30°C	7.2	0	FW	++
DI-34	Chlorococcum	June 1984	28°C	7.8	18	SW	+++
DI-160	Hantzschia	June 1984	29°C	8.0	26	SW	++++
TR-67	Scenedesmus	June 1984	29°C	7.6	0	FW	++
TR-84	Scenedesmus	June 1984	29°C	7.6	0	FW	++++
TR-87	Ankistrodesmus	June 1984	29°C	7.6	0	FW	++++
TR-114	Nitzschia	June 1984	32°C	7.4	0	FW	++++
TL-24	Ankistrodesmus	July 1984	32°C	7.4	0	FW	++
TR-40	Chlorella, Flagellate	July 1984	30°C	7.6	0	FW	+
TR-43	Selenestrum, Closterium	July 1984	30°C	7.6	0	FW	++
TR-45	Scenedesmus, Closterium	July 1984	30°C	7.6	0	FW	++
UP-61	Unicellular (green)	July 1984	32°C	7.4	0	FW	+
UP-62	Motile (green)	July 1984	32°C	7.4	0	FW	+
UP-87	Filamentous (green), Unicellular (green)	July 1984	32°C	7.4	0	FW	+

1. Site Collection

AR = Alabama River
BW = Black Warrior River
DI = Dauphine Island
MB = Mobile Bay
MF = Miller Ferry Reservoir
SL = Smith Lake
SR = Spisey River
TL = Tuscaloosa Lake
TR = Tombigbee River
UP = University Pond

2. Growth Condition

FW = Freshwater
SW = Saltwater

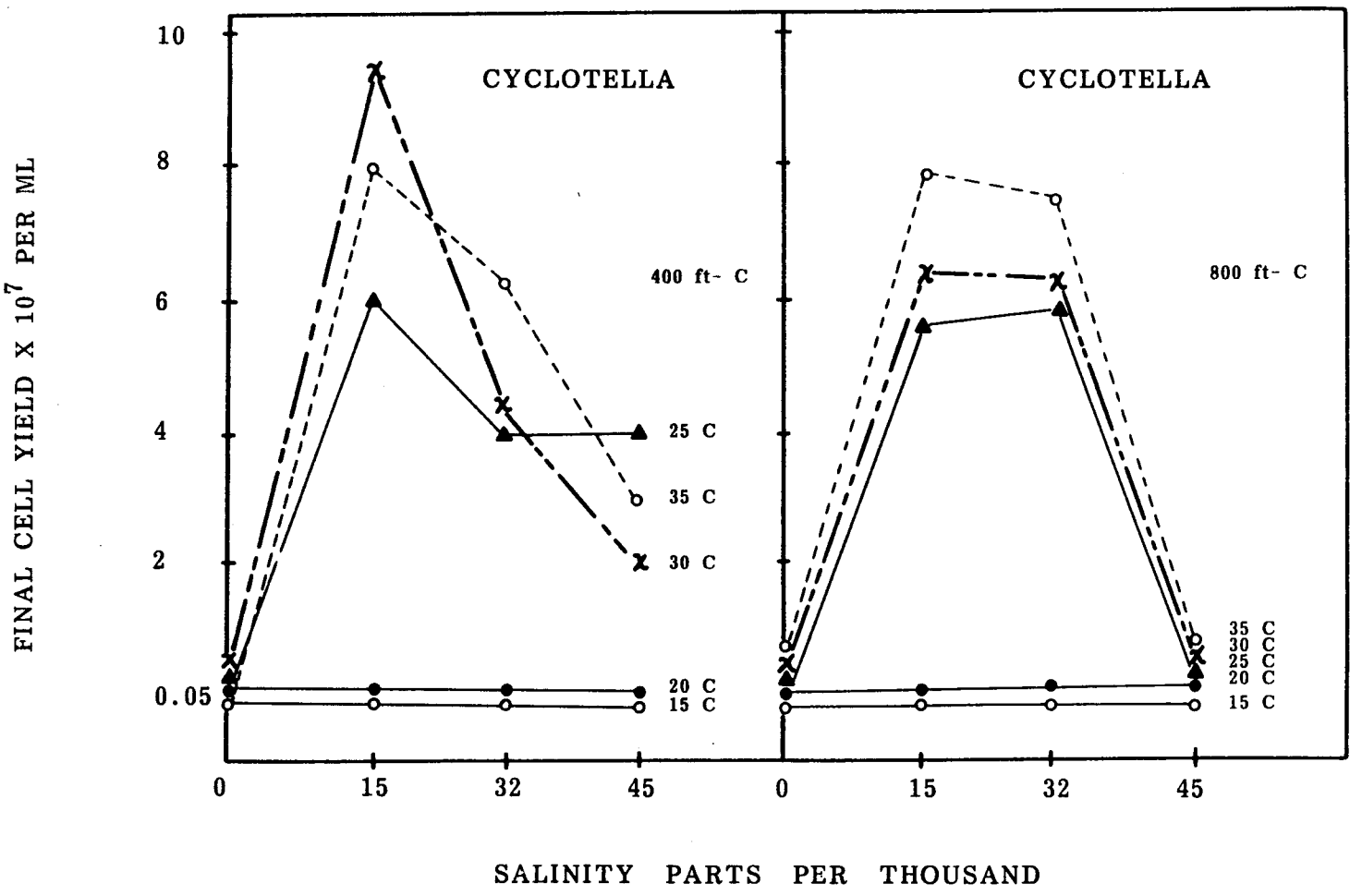

Figure 3. **Final** Yield of Cyclotella sp. as a Function of Temperatures, Light Intensity and Salinity, after 12 Days Growth Period

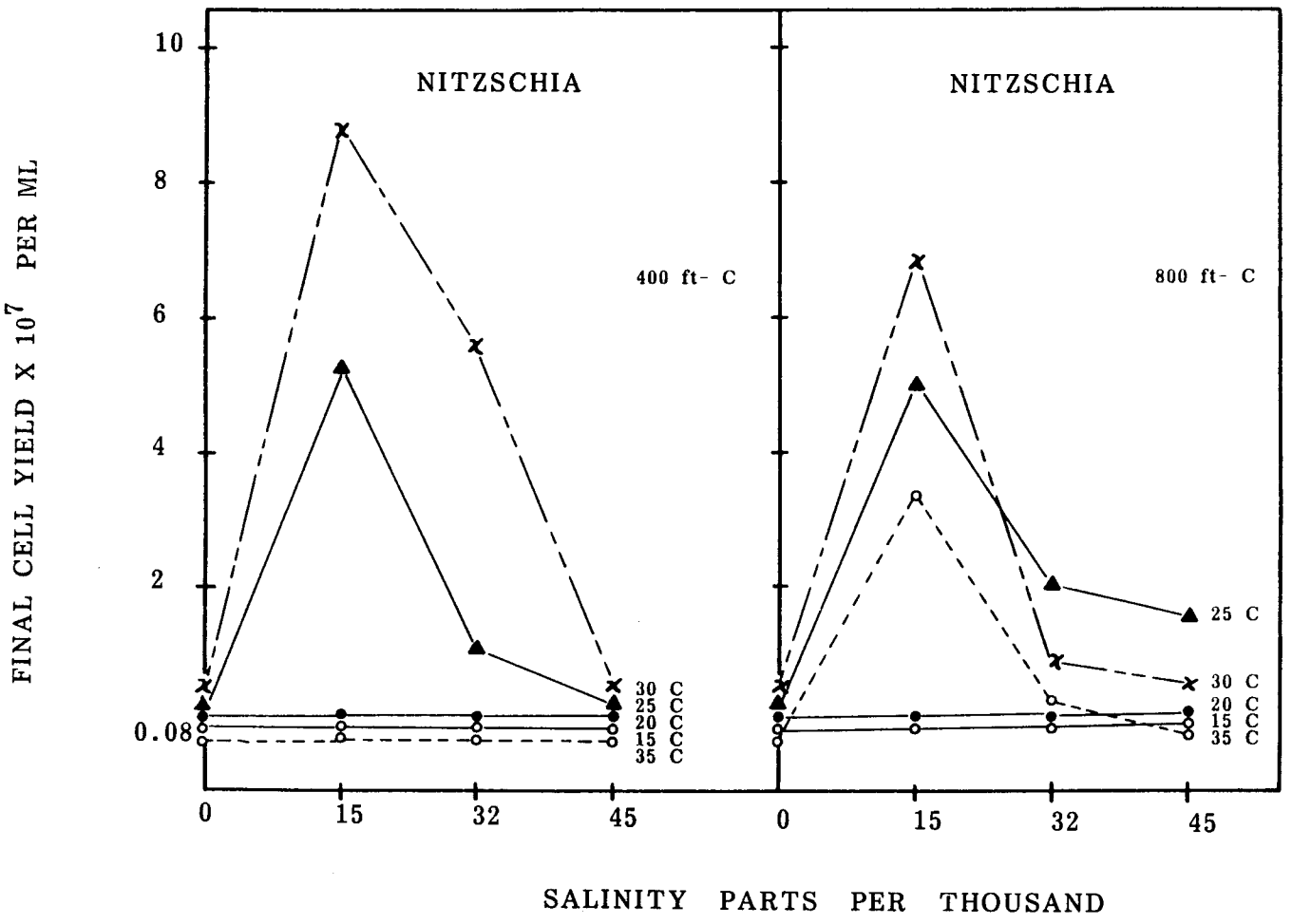

Figure 4. Final Yield of Nitzschia sp. as a Function of Temperature , Light Intensity and Salinity, after 12 Days Growth Period

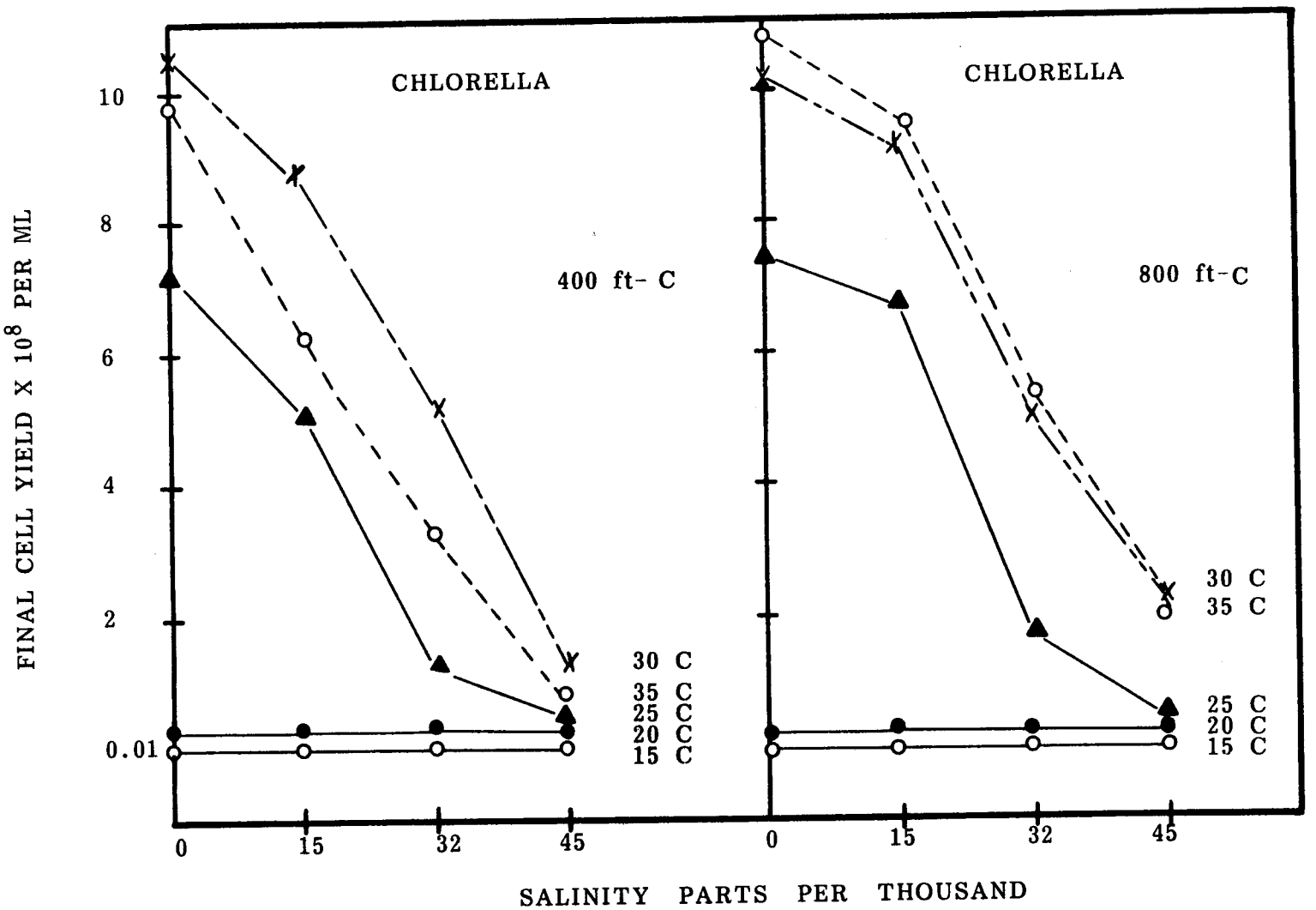

Figure 5. Final Yield of Chlorella sp. as a Function of Temperature , Light Intensity and Salinity, after 12 Days Growth Period

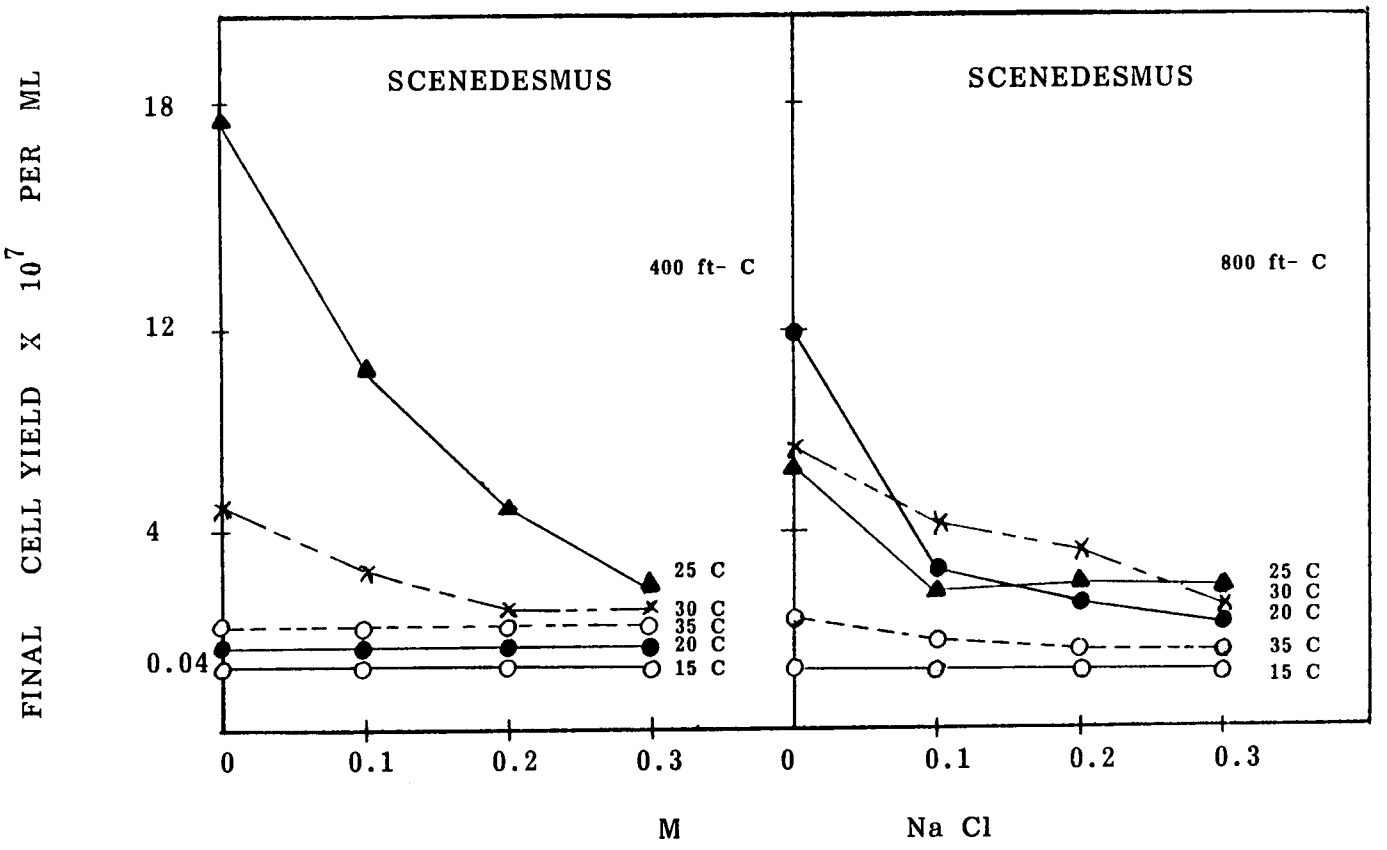

Figure 6. Final Yield of Scenedesmus sp. as a Function of Temperature , Light Intensity and Sodium Chloride Concentration , after 12 Days Growth Period

Temperature, Light, Salinity Requirements

The growth parameters for the selected species were examined under different combinations of light intensity, temperature and salinity, in order to define the optimal growth conditions as well as high and low limits. The gradient block was used for this experiment. The maximum densities reached by each species was determined by evaluating cell counts in the stationary phase. The results are represented in Figures 3, 4, 5, 6, 7.

Cyclotella sp.: (Fig. 3)

An increase in cell number could not be detected at 0.0 ppt. salinity; however increasing the salinity to 15 ppt. resulted in an increase in final culture density. At 32 ppt. salinity, the maximum yield of cells declined and reached about 75% that at 15 ppt. salinity. At both light intensities, 400 ft-C and 800 ft-C, the yield decreased in higher salinities. High temperatures, 30°C and 35°C under 400 ft-C and 800 ft-C light intensities, favored maximum yield of cells. On the other hand, at lower temperatures (15°C and 20°C) cell division completely ceased. It is clear from these results that Cyclotella sp. DI-35 tolerates high temperatures (30°C, 35°C) and salinities (32 ppt.) at high light intensity (800 ft-C). Therefore, growth conditions for Cyclotella sp. DI-35 are: Temperature: 25°C - 35°C; Light intensity: 400 ft-C - 800 ft-C; Salinity: 15 ppt. - 32 ppt.

Nitzschia sp. TR-114: (Fig. 4)

The optimum yield of cell division was obtained at 15 ppt. salinity and 30°C and 400 ft-C. At 25°C, no obvious effect of light intensity was observed. Lower temperatures (20°C and 15°C) arrested the growth of the diatom. Therefore, growth conditions for Nitzschia sp. TR-114 are: Temperature: 20°C - 35°C; Light intensity: 400 ft-C - 800 ft-C; Salinity: 15 ppt. - 32 ppt.

Chlorella sp. MB-31: (Fig. 5)

This species grew in 0.0 ppt. as well as 15 ppt. salinities. Higher yield was favored by 800 ft-C and 35°C. Lower temperatures (15°C, 20°C) arrested completely the growth of the alga at all salinities. Growth conditions for Chlorella sp. MB-31 are: Temperature: 25°C - 35°C; Light intensity: 400 ft-C - 800 ft-C; Salinity: 0.0 ppt. - 32 ppt.

Scenedesmus sp. TR-84: (Fig. 6)

The final cell concentration was highest at 400 ft-C light intensity and 25°C. It decreased as the temperature increased to 30°C and 35°C. Increasing sodium chloride concentration in the growth medium produced an inhibitory effect at both light intensities. Growth conditions for Scenedesmus sp. TR-84 are: Temperature: 25°C - 30°C; Light intensity: 400 ft-C - 800 ft-C; Salinity: 0.0 - 0.2 M NaCl.

Ankistrodesmus sp. TR-87: (Fig. 7)

High light intensity (800 ft-C) and temperature (30°C) favored higher cell division rates. Sodium chloride exhibited an inhibitory effect when increased to 0.2 M. Environmental conditions producing growth in Ankistrodesmus sp. TR-87 were: Temperature: 20°C - 35°C; Light intensity: 400 ft-C - 800 ft-C; Salinity: 0.0 - 0.2 M NaCl.

From the results reported in this experiment, it is evident that estuarine species as Cyclotella sp.; Nitzschia sp.; Chlorella sp. have the ability to tolerate wide salinities from 15 ppt. to 32 ppt. and temperatures from 20°C to 35°C. On the other hand, the freshwater species such as Scenedesmus sp. and Ankistrodesmus sp. respond significantly to sodium chloride concentrations and their yield drops drastically.

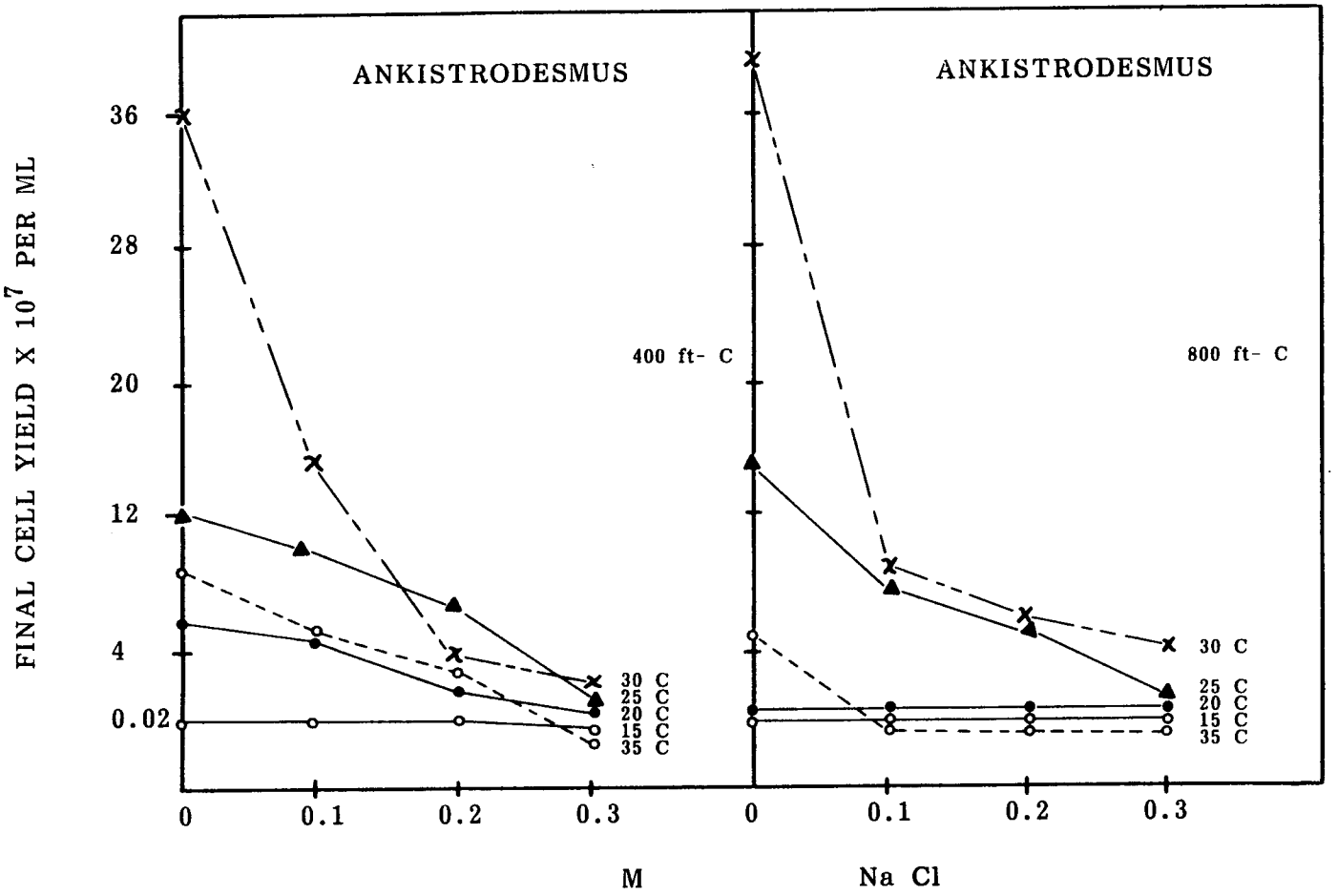

Figure 7. Final Yield of Ankistrodesmus sp. as a Function of Temperature , Light Intensity and Sodium Chloride Concentration , after 12 Days Growth Period

Nitrogen Source Requirements

The selected strains were treated with different concentrations of urea and nitrate (see Methods). The growth response of the strains, was evaluated by growth curves (Figures 8, 9, 10, 11, 12).

In the diatom (Cyclotella sp. DI-35 (Fig. 8), the cell number increased with increasing the urea - N (1mM). As the urea - N was increased to 5 mM, the cell number dropped. Similar results were obtained with nitrate-N. It enhanced the cell number at 2 mM and inhibited it at 5 mM concentrations. In the case of Nitzschia sp. TR-114 (Fig. 9), cultures containing 0.01 mM nitrate-N reached greater cell densities than those containing 2 mM nitrate-N. Urea - N, compared to nitrate-N, produced no significant effect on the growth of Nitzschia sp.

For the Chlorophyceae: Chlorella sp. MB-31; Scenedesmus sp. TR-84; Ankistrodesmus sp. TR-87; results are represented in Figures 10, 11, 12. The growth curves showed similar responses to N concentrations. Increasing urea and nitrate concentrations in the medium resulted in higher cell densities. However, in all growth curves, the final yield of cell number was still limited by N concentration in the medium. Increasing urea and nitrate concentrations could produce higher cell numbers. Ammonium chloride as a N source caused cellular bleaching in all strains, therefore the results were not recorded.

It can be concluded that N-source requirements vary according to the species. Diatoms utilize nitrate rather than urea, while the growth of unicellular green species enhanced more by urea. This is in agreement with Reimann, et. al. (1963) who reported that Cyclotella cryptica species were unable to grow on urea. In addition, growth of diatoms was not limited by N concentration. By varying N concentrations in the nutrient medium, all the green algal species investigated could be manipulated with respect to their biomass production. In order to obtain higher yields, the green algal species could presumably be grown at still greater N concentrations.

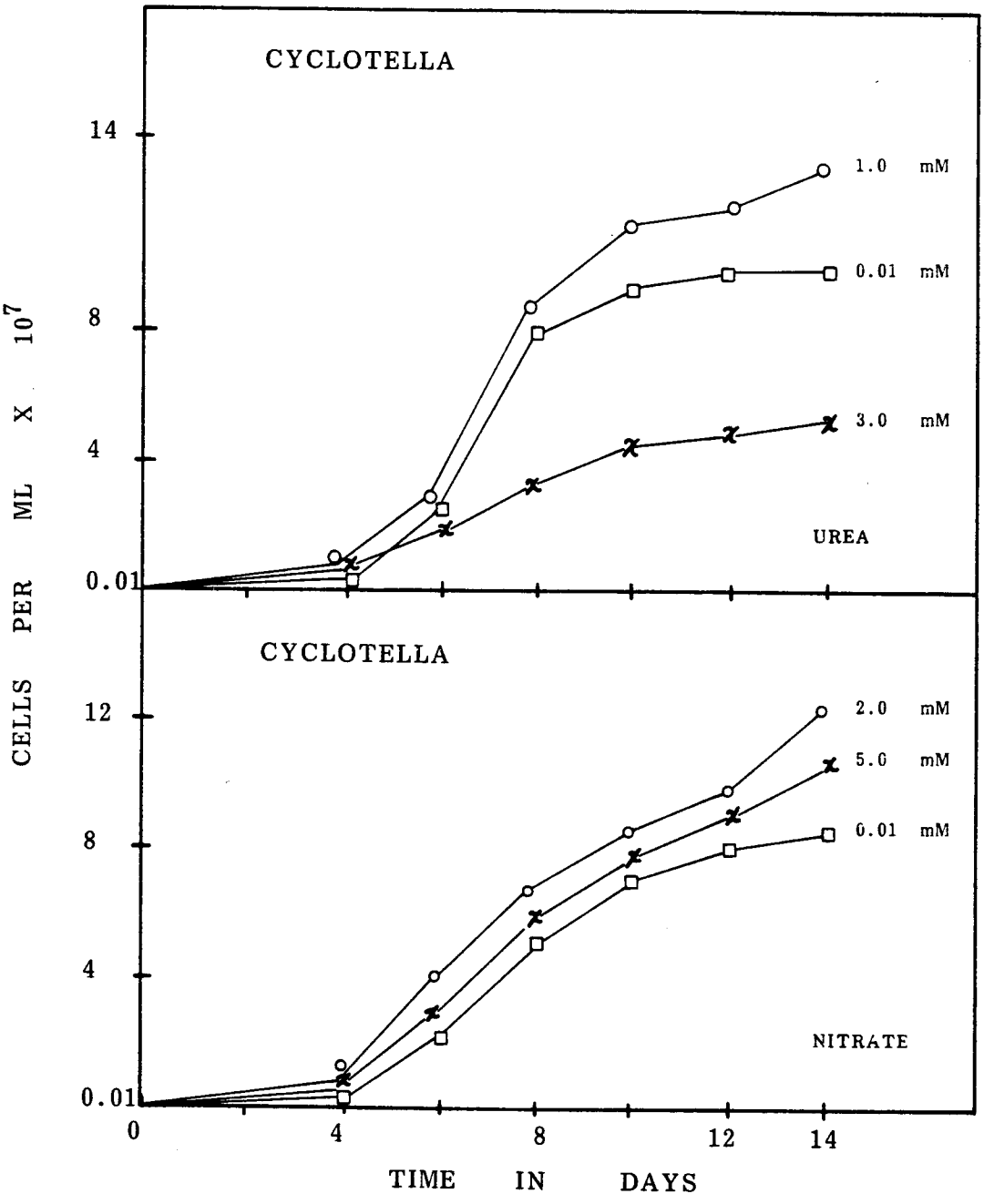

Figure 8. Growth Curve of Cyclotella sp. in a Series of Media Containing Different Concentrations of Urea or Nitrate (as N-source).

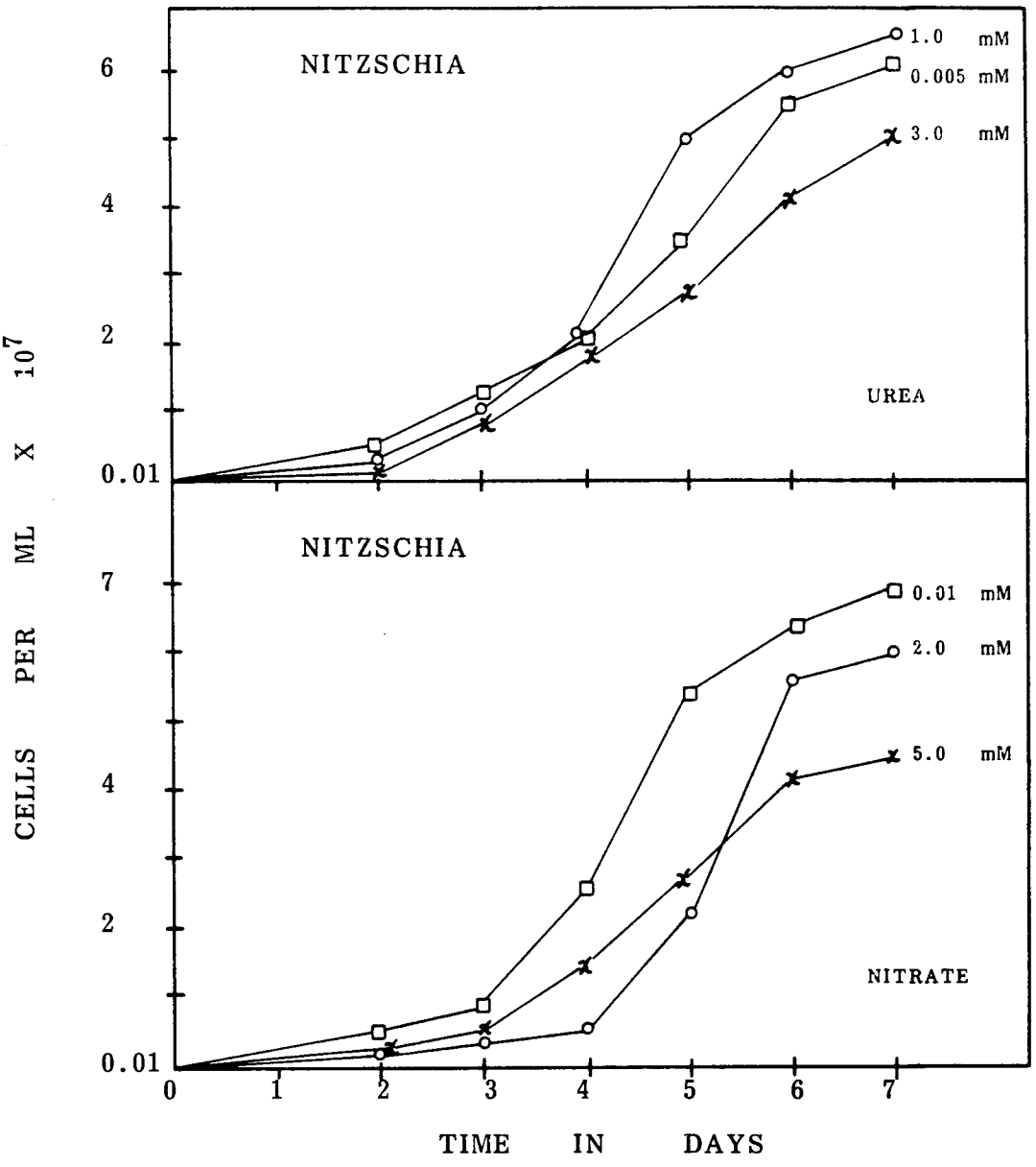

Figure 9. Growth Curve of Nitzschia sp. in a Series of Media Containing Different Concentrations of Urea or Nitrate (as N-source)

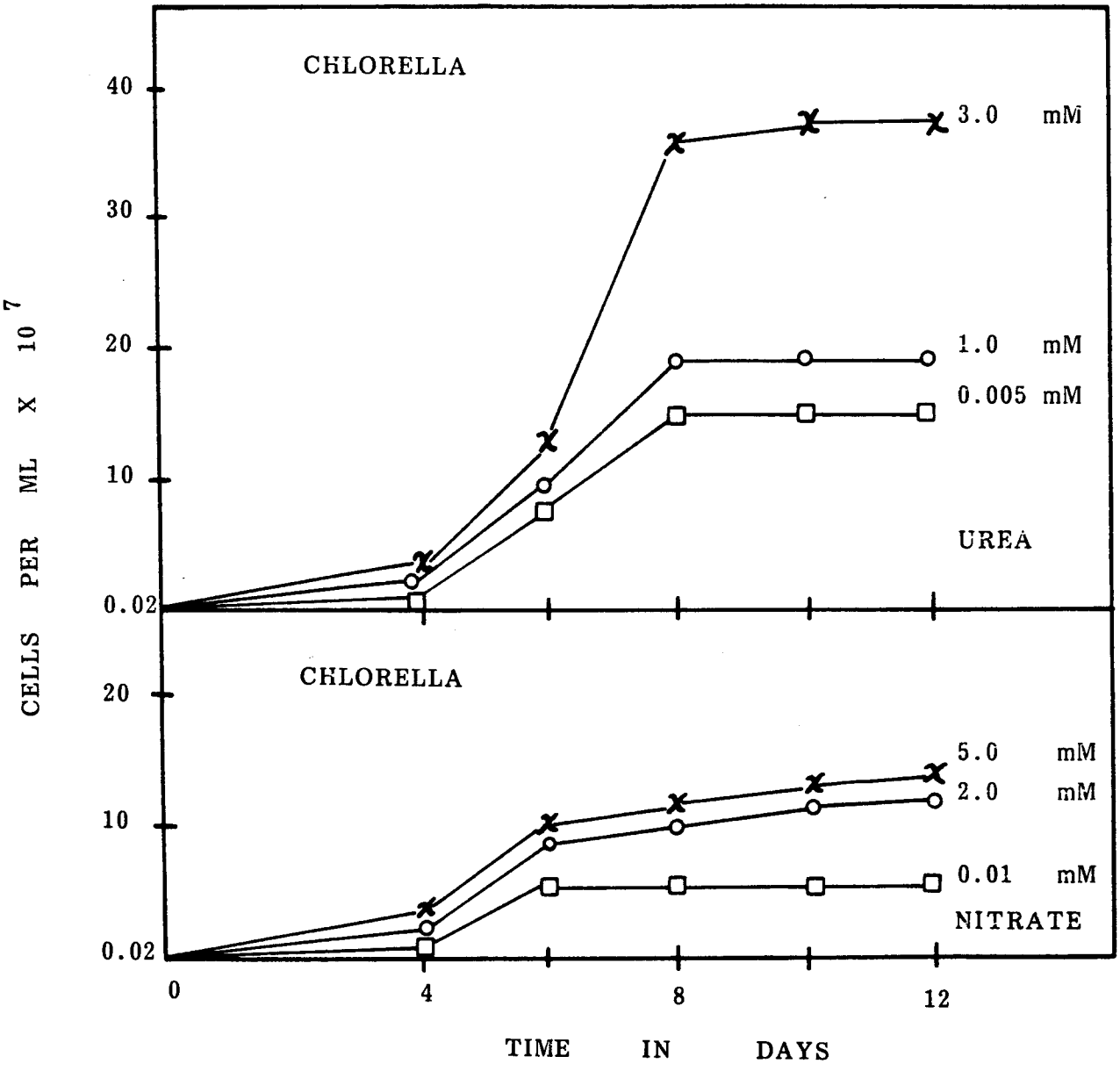

Figure 10. Growth Curve of Chlorella sp. in a Series of Media Containing Different Concentrations of Urea or Nitrate (as N-source)

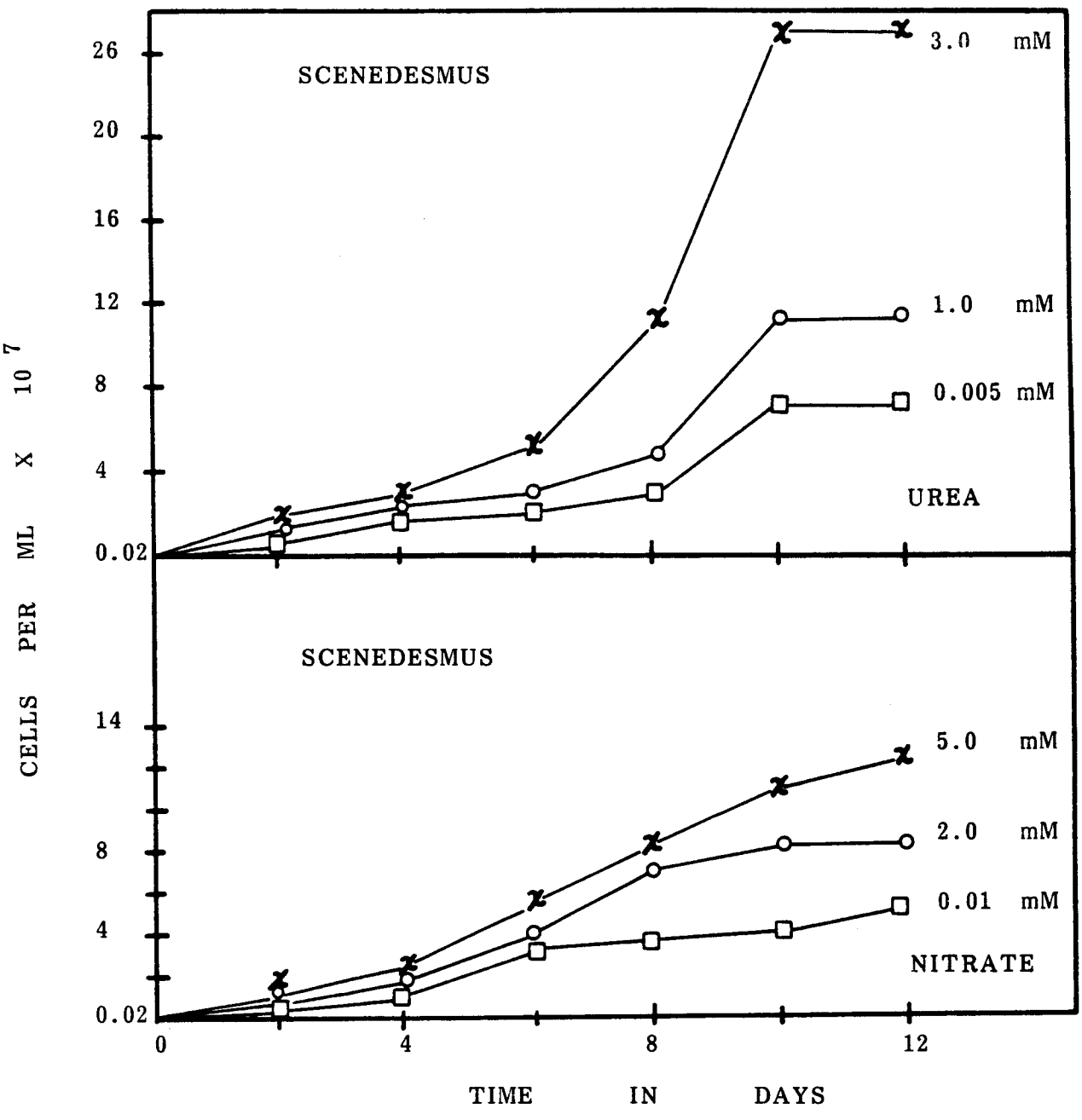

Figure 11. Growth Curve of Scenedesmus sp. in a Series of Media Containing Different Concentrations of Urea or Nitrate (as N-source)

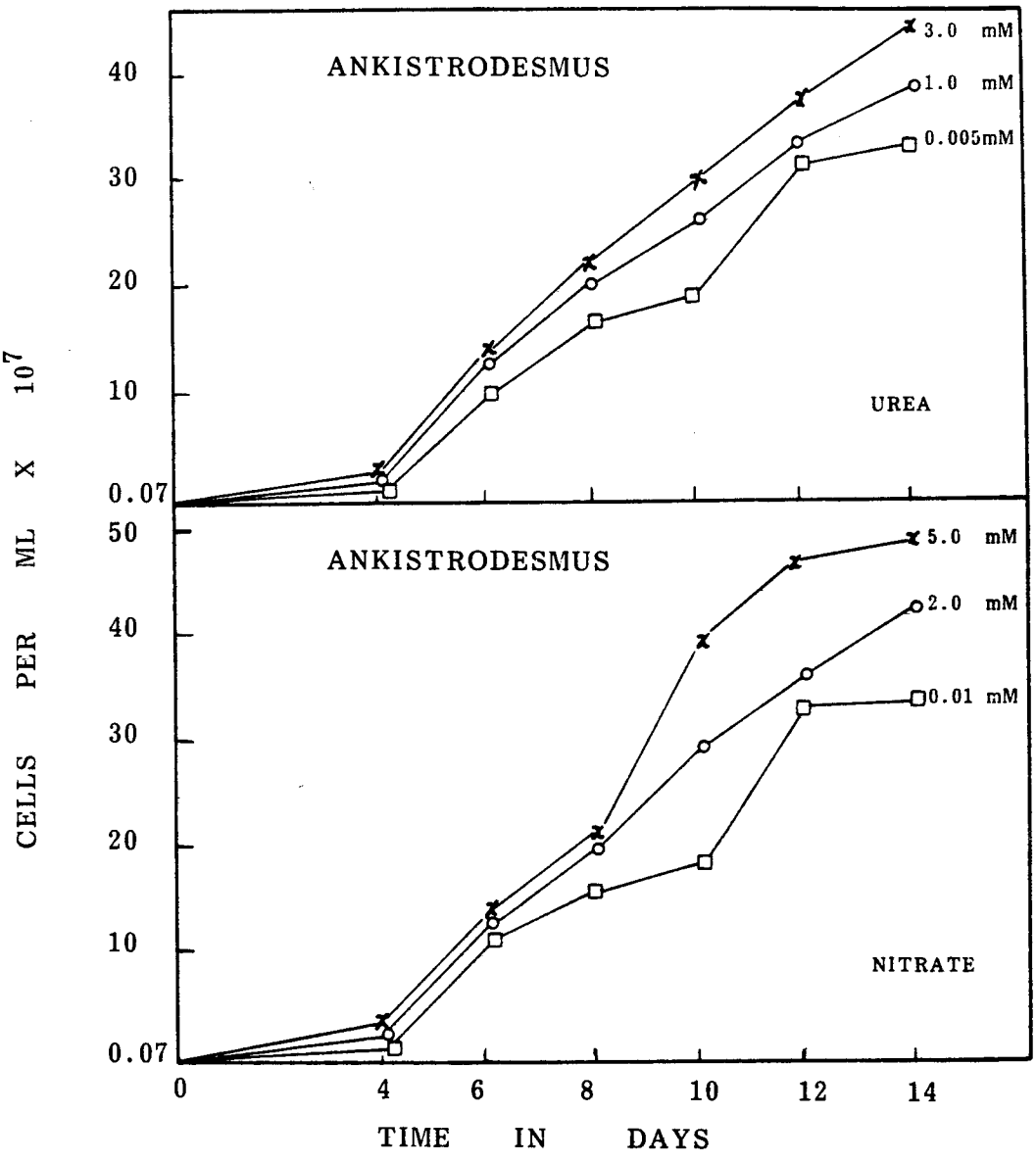

Figure 12. Growth Curve of Ankistrodesmus sp. in a Series of Media Containing Different Concentrations of Urea or Nitrate (as N-source)

Approximate Analysis for the Selected Species

In this experiment, all the selected species were grown in duplicate batch cultures, as previously described. The cultures were maintained under similar temperatures and light intensities. One batch of each species was analyzed in the exponential growth phase (5 days old), when the cells were actively dividing and sufficient nitrogen was available in the medium. The second batch was analyzed in the stationary phase (14 days old), when the cells ceased dividing and the medium had become N-depleted. Data for approximate cellular compositions were expressed on the basis of organic weight and represented in Table 2. In Cyclotella, N-deficient cells contained 42.1% total lipids. Nitzschia, on the other hand, under N-stress contained 28.1% lipids. Chlorella, whether in freshwater or saline medium, did not show a clear difference in composition. Nevertheless, N-starved cells contained more lipids 28.6% (freshwater) and 32.4% (saline) than N-sufficient cells which contained 15.3% and 26.5% respectively. Proteins decreased on the expense of carbohydrates which increased relatively. Nitrogen deficient cells of Scenedesmus synthesized almost 44.7% total lipids, almost double that of the young cells. In addition, N-exhausted cells developed an orange color resulting from the accumulation carotenoid pigments. In experiments with the Ankistrodesmus sp. the total lipids reached up to 28.1% in N-depleted cultures.

An interesting diatom, identified as Hantzschia sp. DI-160 by Dr. Barclay at SERI, was collected from the Dauphin Island. Although the culture was not unialgal, it grew very well in enriched saltwater (salinity 45 ppt.). Growth requirements of this species have not yet been identified. However, approximate analysis (Table 2) revealed the presence of high amounts of lipids (66%). The cover sheet of this report represents a picture of this species containing oil droplets.

It should be mentioned that in case of diatoms, the cells accumulate oil droplets as a result of N-deficiency. Silicon efficiency was studied independently for these strains to assure that oil formation was due to N-deficiency and not to silicate depletion. In case of the green algal strains, the cells changed in color to orange or yellow in the stationary phase, as a result of N-depletion.

The most general effect of a nitrogen deficiency on the composition of the selected strains is a decrease in protein and an increase in the storage products, lipids and carbohydrates. The results are in agreement with those of Fogg, 1953 and Healy, 1973.

FUTURE PLANS

Specific research plans for FY 85 - FY 86 will follow the research objectives as explained above in this report, with special emphasis:

° Sample collections from saline habitats in the Gulf of Mexico

° Screening of the samples under higher light intensities (20 - 50% of sunlight).

Table 2. Approximate Cellular Composition of Selected Algal Species

Species	Cell Size (μM^3)	Growth Rate	Growth Conditions	% Organic Wt. Protein	% Organic Wt. Carbohydrate	% Organic Wt. Lipid
Cyclotella sp.			SW, NE	12.2	37.5	13.2
DI-35	3-5	1.37	SW, ND	16.4	10.2	42.1
Nitzschia sp.						
TR-114	10-15	0.84	SW, NE	25.7	18.8	15.2
			SW,ND	7.2	13.2	28.1
Chlorella sp.	2-3	0.92	FW, NE	51.2	12.3	15.3
MB-31			FW, ND	25.4	26.2	28.6
			SW, NE	23.5	24.7	26.5
			SW, ND	18.3	29.6	32.4
Scenedesmus sp.	5-6	1.79	FW, NE	30.2	29.8	20.3
TR-84			FW, ND	11.4	32.3	44.7
Ankistrodesmus sp.	5-7	1.11	FW, NE	35.3	32.5	16.9
TR-87			FW, ND	24.5	38.2	28.11
Hantzschia sp.	15-35	1.32	SW, NE	20.2	29.4	26.3
DI-60			SW, ND	12.6	9.3	66.0

FW = Freshwater SW = Saltwater NE = Nitrogen Sufficient ND = Nitrogen Deficient

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Collection trips were made to Dauphin Island near the Alabama coast during the summer of 1984. Over sixty strains were isolated, and of these six were ranked as good growers. Two diatoms were isolated that are of particular interest because of their ability to accumulate high lipids. Cyclotella tolerates high temperatures (30°-35°C), grows at moderate salinities (15-25 parts per thousand), and with nitrogen stress accumulates 42% of its dry weight as lipid. Hantzschia is a large diatom that also grows well at elevated temperatures and full strength seawater. Hantzschia can accumulate as much as 66% of its dry weight as lipid.

17. Document Analysis
a. Descriptors Alabama; Algae; Chlorella; Lipids; Salinity; Scenedesmus; Unicellular algae

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