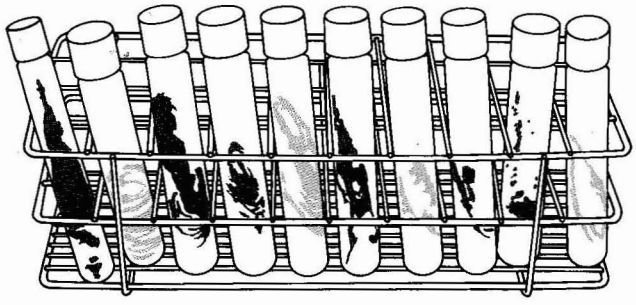
SERI/SP-232-2863
UC Category: 61c



Microalgae Culture Collection 1985-1986

January 1986

Prepared by the
Microalgal Technology Research Group

Solar Energy Research Institute
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1617 Cole Boulevard
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Prepared for the
U.S. Department of Energy
Contract No. DE-AC02-83CH10093

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INTRODUCTION

In 1984, the SERI Microalgae Culture Collection was established in support of the U.S. Department of Energy's Biofuels Program. It provides a repository for strains identified or developed for mass culture biomass production and makes these strains readily available to the research community. The strains in the collection have been selected for their potential in biomass fuel applications, and many produce significant quantities of cellular storage lipids.

The 1984-1985 Culture Collection Catalog listed twelve strains of ten species. An additional ten strains have been included in the 1985-1986 catalog. All of the newly added strains have been recently isolated by SERI and its subcontractors in focused screening programs. Many have been tested in outdoor mass culture systems, and several have demonstrated excellent performance as biomass producers, with yields of up to 40 grams of organic matter per square meter per day. The majority of strains added to the collection this year have been isolated from inland saline waters, although marine species are included as well. We believe that the strains in this collection can provide a source of extremely useful organisms, both for laboratory experimentation and for mass culture research.

The response of the research community to the first catalog was excellent; over 100 cultures were shipped to investigators with diverse interests including fuel production, photosynthesis research, natural product screening, ultrastructural studies, and aquacultural feedstock research. **Again this year, cultures will be shipped free of charge to interested researchers.** As we did last year, we request that investigators using species from this collection supply us with copies of pertinent publications of their research with these strains.

An important function of the culture collection catalog, in addition to listing the available strains, is to provide culture and performance data for each of the organisms. By collecting a summary of the requirements and characteristics of these organisms, we hope to allow requestors of cultures to begin productive research with a minimum of preliminary work on culture techniques. In the previous catalog, we also provided a listing of references available in a data base for each species. However, since only a few requests for this data base information were received, this service will not be offered this year.

Microalgal research at SERI focuses on biomass energy production: thus, the SERI culture collection is limited to those clones or strains that (1) have high potential as a fuel feedstock (lipid and carbohydrate producers), and (2) have been at least partially characterized for culture requirements and chemical composition. The criteria that guide the selection of clones or strains for the collection, in descending order of importance, are as follows:

- Energy yield (growth rate x energy content)
- Type of fuel products available from biomass (hydrocarbon, diesel, alcohol, methanol)
- Environmental tolerance range (temperature, salinity, pH)

- Performance in mass culture (highly competitive, predator resistant)
- Media supplementation requirements (addition of vitamins, trace minerals)
- Amount of culture and composition data available on the clone or strain
- Budget for the culture collection.

A steering committee is convened once a year to review new strains for addition to the collection according to these criteria. This year, we gratefully acknowledge the participation of Dr. Ian Morris, Dr. Craig Sandgren, and Mr. Robins McIntosh.

Explanatory Notes

Although most of the data listed in the summary sheets are self explanatory, details concerning some of the data are as follows:

Available nitrogen sources. The nitrogen sources listed are known to be satisfactory; other forms may also be available to the alga.

Suitable media. Formulas for suitable culture media for each strain are listed in the Appendix.

Chemical composition. Symbols used are as follows:

Growth conditions:	B	batch culture
	C(X)	continuous culture (X = specific growth rate, day^{-1})
	SC	semicontinuous culture
	MC	outdoor mass culture
	N(X)	nutrient limited, where X is replaced by P for phosphorus limitation, N for nitrogen limitation, or C for carbon (CO_2) limitation
	L(n)	light limited, where n is the culture irradiance in μEinst m^{-2} s^{-1}
Basis:	C	carbon
	DW	dry weight
	AFDW	ash-free dry weight

Lipid composition data in some cases are summarized as the fraction of lipids extracted by one of five solvents, in a serial extraction process running from hexane to methanol. The composition of the various fractions is as follows: hexane fraction = acyclic hydrocarbons; benzene fraction = isoprenoids; chloroform fraction = tri-, di-, and monoglycerides, free fatty acids; acetone fraction = glycolipids; and the methanol fraction = phospholipids.

Fuel Options. Each of the three biochemical fractions (lipids, carbohydrates, and proteins) can be converted into fuels. Lipids, with the highest energy content of the three, can be converted into a fuel similar to diesel oil by the process of transesterification. Carbohydrates are commonly converted to ethanol by fermentation. Alternatively, all three fractions can be converted to methane gas by anaerobic digestion. Fuel production options were calculated for each strain based on its chemical composition under nutrient limited conditions. The assumptions and procedures for these calculations have been outlined in ***Fuel Options from Microalgae with Representative Chemical Compositions*** (by D. Feinberg, Solar Energy Research Institute, SERI/TR-231-2427, 1984). This report first presents the gross energy content available from a unit mass of each strain and then five options to convert each fraction into fuel products. The five options listed in the summary tables are: Option 1 - methane production by anaerobic digestion of the entire ash-free cell mass; Option 2 - methane production by anaerobic digestion of the cell mass, excluding glycerol which is sold as a by-product; Option 3 - production of methane and ester fuels by digestion of the protein and carbohydrate fractions only, with lipids being converted to ester fuels and hydrocarbons; Option 4 - production of ethanol and methane by digestion of the lipid and protein fractions, with the carbohydrate converted to ethanol; and Option 5 - production of methane, ethanol, and ester fuels by digestion of the protein fraction only, with ester fuel and ethanol production from the lipid and carbohydrate fractions, respectively.

Requests for Cultures

All cultures in this catalog are available without charge for research and culture applications. Requests for cultures are accepted by letter, which should be addressed as follows:

Dr. Bill Barclay
Microalgae Culture Collection
Solar Energy Research Institute
FTLB
1617 Cole Blvd.
Golden, CO 80401

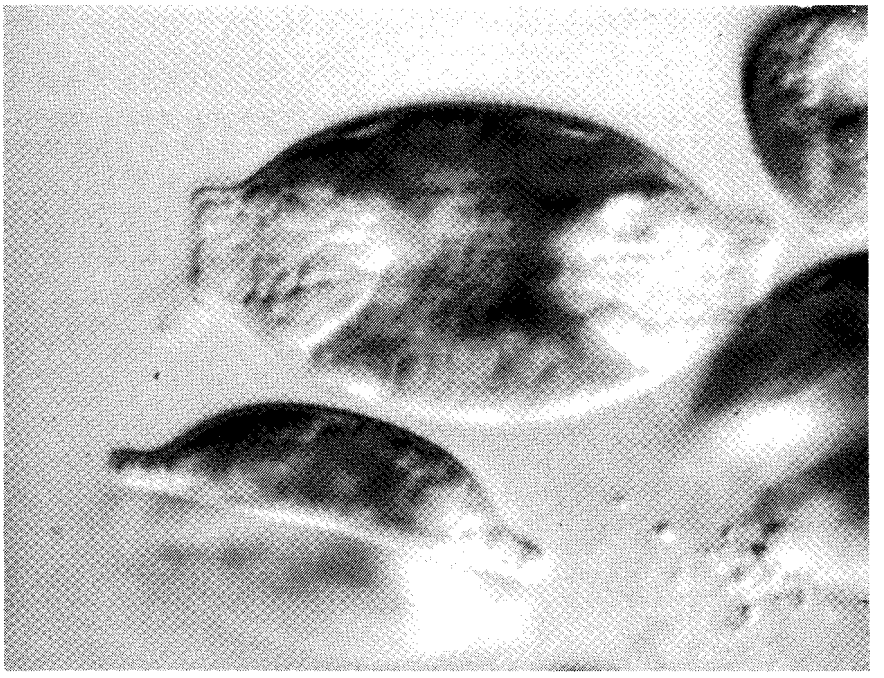
Questions about the culture collection or requests for information can be made by phone to (303)231-1842.

We request that investigators using species from this collection please send us copies of publications resulting from research on these strains.

Amphora sp.

Strain: S/AMPHO-1

Taxonomy: **Division:** Chrysophyta
Class: Bacillariophyceae
Order: Pennales
Family: Cymbellaceae



Cells of *Amphora* sp. S/AMPHO-1 (Scale: 1 cm = 3.8 μm)

Collection site: Glenwood Springs, Colorado, USA (W. Barclay)

Date: November 1984
Water temperature: 33°C
Salinity: 34 mmho cm^{-1} conductivity
pH: 7.6

Size: 20-30 μm

Growth form: unicells

Growth rate at optimum (or maximum recorded): 5.1 doublings day^{-1} (1)

Culture conditions:

Vitamins required: not determined

Available nitrogen sources: urea

Suitable media: SERI Types I, II, also RILA

Nutritional modes: autotrophic

Temperature range: 20°>35°C

Salinity range: <10->70 g TDS L^{-1}

Chemical composition:

Growth Conditions	% lipid	% protein	% carbohydrate	Ref.	Basis
MC	20			1	AFDW
B	4.1	30.1	36.2	2	AFDW
B, N(N)	10.2	20.6	70.1	2	AFDW
B, N(N, severe)	13.6	17.3	74.9	2	AFDW

Fuel production options:

Option	Methane MJ/kg	Ester MJ/kg	Hydro-carbon MJ/kg	Ethanol MJ/kg	Total Recovered MJ/kg	Fraction Utilized	Liquid Fuel MJ/kg
1	13.7	0	0	0	13.7	0.693	0
2	13.7	0	0	0	13.7	0.693	0
3	10.6	3.12	0	0	13.7	0.694	3.12
4	6.0	0	0	6.9	12.9	0.650	6.86
5	2.9	3.1	0	6.9	12.9	0.652	9.99

Total energy content: 19.8 MJ/kg dry weight

Physiological notes:

1. Growth at pH range 7-10. (1)

2. Highly tolerant of high concentrations of dissolved oxygen. At 500% O_2 saturation relative to air, growth is reduced only 5% below that of a population maintained at equilibrium. (1)

Life cycle:

Vegetative cell division is the ordinary method of reproduction. Sexual reproduction is isogamous in *Amphora* spp. Amoeboid gametes fuse resulting in the formation of an auxospore.

Outdoor culture history:

This strain was cultured outdoors for three weeks during July and August 1985, at Vacaville, California. Productivity was as high as 45-50 g DW m^{-2} d^{-1} in SERI Type I medium at a low salinity maintained at pH 7.5-8.0. The average productivity over three weeks of growth was 30 g DW m^{-2} d^{-1} (6.8% photosynthetic efficiency on PAR). (1)

Literature cited:

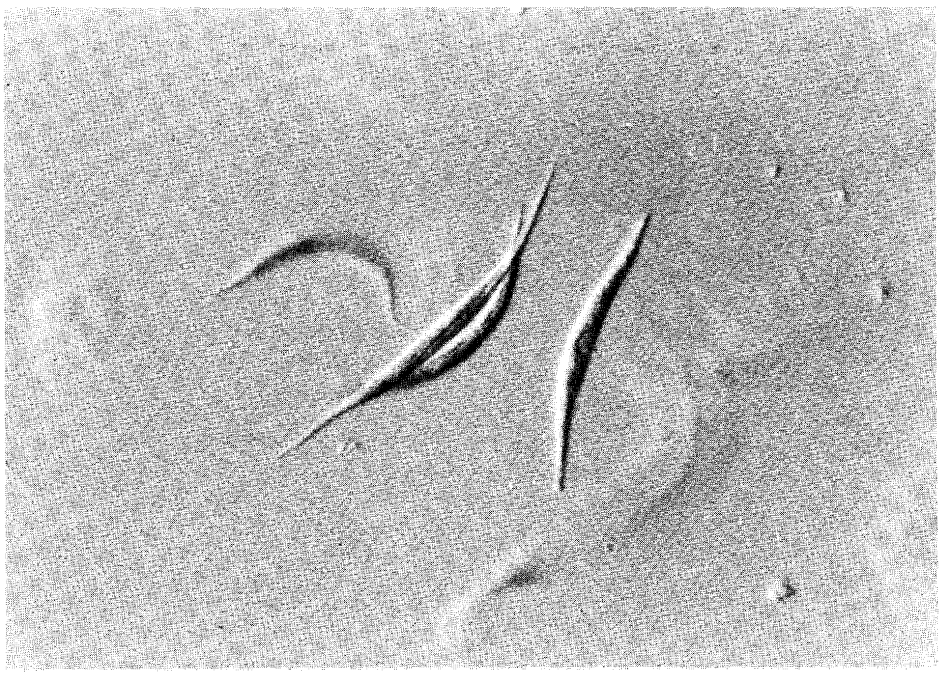
1. Weissman, J. Unpublished data.

2. Benemann, J. Unpublished data.

Ankistrodesmus falcatus

Strain: S/ANKIS-1 (Pyramid Lake, 91-1)

Taxonomy: **Division:** Chlorophyta
Class: Chlorophyceae
Order: Chlorococcales
Family: Oocystaceae



Cells of *Ankistrodesmus falcatus* S/ANKIS-1 (Scale: 1 cm = 10 μm)

Collection site: Pyramid Lake, Nevada, USA (W. Thomas) (1)

Date: October 1982
Water temperature: 17°C
Salinity: 5 g TDS L^{-1}
pH: 9.1

Size: 35-57 μm x 3 μm

Growth form: unicells

Growth rate at optimum (or maximum recorded): 2.89 doublings day^{-1} (3)

Culture conditions:

Vitamins required:	none
Available nitrogen sources:	urea, nitrate, ammonium
Suitable media:	Pyramid Lake
Nutritional modes:	photoautotrophic
Temperature range:	18°-31°C (1)
optimum:	26°C (1)
Salinity range:	1-10 g TDS L^{-1} (1)
optimum:	7 g TDS L^{-1} (1)

Chemical composition:

Growth Conditions	% lipid	% protein	% carbohydrate	Ref.	Basis
B	24.5	31.1	10.8	3	AFDW
B, N(N)	40.3	14.3	18.3	3	AFDW
B, N(C)	19.5	28.6	9.2	4	DW

Lipid composition:

Growth Conditions	Fraction eluted by:					Ref.
	Hexane	Benzene	Chloroform	Acetone	Methanol	
B	0.7	2.5	9.8	72.6	14.4	3
B, N(N)	<0.1	3.3	13.5	66.5	16.1	3
B, N(C)	--	5.8	14.1	66.8	10.5	4

Fuel production options:

Option	Methane MJ/kg	Ester MJ/kg	Hydro-carbon MJ/kg	Ethanol MJ/kg	Total Recovered MJ/kg	Fraction Utilized	Liquid Fuel MJ/kg
1	16.6	0	0	0	16.6	0.753	0
2	16.6	0	0	0	16.6	0.753	0
3	4.1	3.5	0.3	0	7.9	0.360	3.8
4	14.6	0	0	1.8	16.4	0.743	1.8
5	2.1	3.5	0.3	1.8	7.7	0.350	6.6

Total energy content: 22.0 MJ/kg dry weight

Physiological notes:

1. N:P requirement ratio is ~ 21 (mol:mol). (5)*
2. Many strains of *Ankistrodesmus* have low salt tolerance (<10 o/oo). (6)*
3. Ash 7%-14% of dry weight. (4)

Life cycle:

Reproduction is by division of cell into two, four, or eight autospores. Vegetative cells can also form resting cells (aplanospores). (7)

Outdoor culture history:

1. *Ankistrodesmus falcatus* (Pyramid Lake) has been cultivated in circulated ponds in northern California, USA. Optimum temperatures 24°-28°C. Produced 18-20 g m^{-2} d^{-1} at 8-12 o/oo salinity. (8)
2. An unspecified species of *Ankistrodesmus* has been cultured in South Africa for the removal of nitrogen from industrial wastes. (9)

*Data labeled with an asterisk are for other strains of this species.

3. *Ankistrodesmus* sp. was a component of a population grown on diluted pig slurry (liquid phase) in a Dortmund-type system in Northern Ireland. (10)

4. *Ankistrodesmus angustus* and *Ankistrodesmus braunii* have been cultured in troughs in the Soviet Union. These species dominated in spring and fall. Optimum temperatures were 20°-28°C, light 10-20 kilolumens, and production averaged 8-10 g m^{-2} d^{-1}. (11)

Literature cited:

1. Thomas, W.H., D.L.R. Seibert, M. Alden & P. Eldridge. 1984. Cultural requirements, yields, and light utilization efficiencies of some desert saline microalgae. In: *Aquatic Species Program Review: Proceedings of the April 1984 Principal Investigators Meetings.* Solar Energy Research Institute Publication SERI/CP-231-2341. pp. 7-63.

2. Rhee, G.-Y. & I.J. Gotham. 1981. Comparative kinetic studies of phosphate-limited growth and phosphate uptake in phytoplankton continuous culture. *J. Phycol.* 17:257-265.

3. Ben-Amotz, A., T.G. Tornabene & W.H. Thomas. 1985. Chemical profiles of selected species of microalgae with emphasis on lipids. *J. Phycol.* 21: 72-81.

4. Tornabene, T.G. 1984. Chemical profile of microalgae with emphasis on lipids. In: *Aquatic Species Program Review: Proceedings of the April 1984 Principal Investigators Meetings.* Solar Energy Research Institute Publication SERI/CP-231-2341. pp. 64-78.

5. Rhee, G.-Y. & I.J. Gotham. 1980. Optimum nitrogen-to-phosphorus ratios and coexistence of planktonic algae. *J. Phycol.* 16:486-489.

6. Kessler, E. 1977. Physiological and biochemical contributions to the taxonomy of the genera *Ankistrodesmus* and *Scenedesmus.* IV. Salt tolerance and thermophily. *Inst. Bot. und Pharm. Fiziol.* 113:143-144.

7. McMillan, R. 1957. Morphogenesis and polymorphism of *Ankistrodesmus* spp. *J. Gen. Microbiol.* 17:658-677.

8. Weissman, J.C. 1984. Cultivation of microalgae for liquid fuels. In: *Aquatic Species Program Review: Proceedings of the April 1984 Principal Investigators Meetings.* Solar Energy Research Institute Publication SERI/CP-231-2341. pp. 132-150.

9. Hendricks, F. & J. Bosman. 1981. The removal of nitrogen from an inorganic industrial effluent by means of intensive algal culture. *Water Sci. Technol.* 13:TOR651-TOR666.

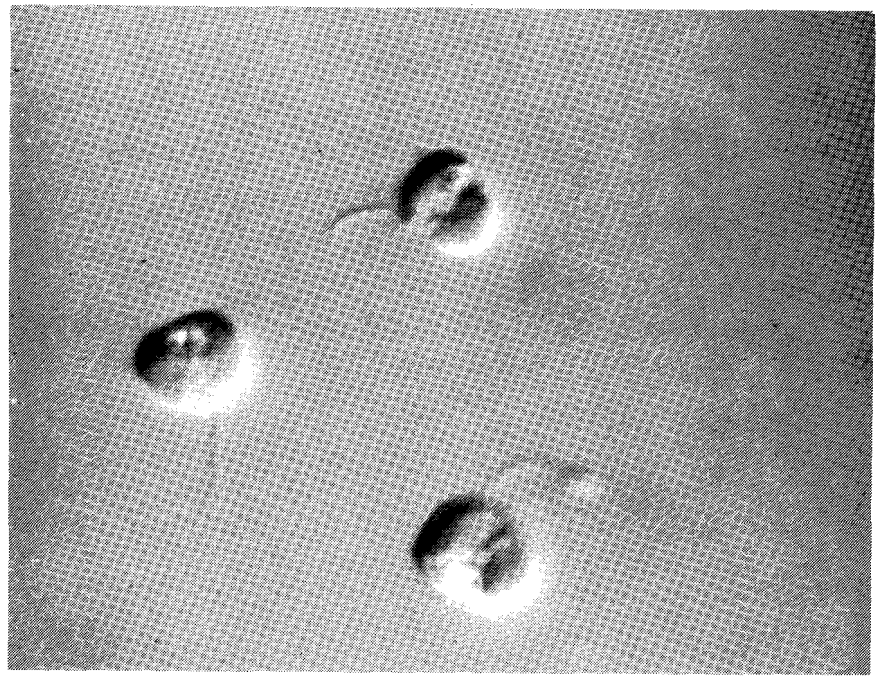
10. Fallowfield, H.J. & M.K. Garrett. 1983. Mass outdoor culture of algae on effluents in Northern Ireland. *Br. Phycol. J.* 18:203.

11. Berdykulova, K.A. & D.A. Nurieva. 1980. Mass cultivation of some microalgae in open settings. *Uzb. Biol. Zh.* 0(3):40-42.

Boekelovia sp.

Strain: S/BOEKE-1

Taxonomy: **Division:** Chrysophyta
Class: Chrysophyceae
Order: Ochromonadales
Family: Ochromonadaceae



Cells of *Boekelovia* sp. S/BOEKE-1 (Scale: 1 cm = 3.3 μm)

Collection site: Saline spring near the junction of Piceance Creek and the White River in northwestern Colorado, USA (W. Barclay)

Date: July 15, 1984
Water temperature: 20°C
Salinity: 10 mmho cm^{-1} conductivity
pH: 9.5 - 10.0

Size: 6 μm

Growth form: unicells

Growth rate at optimum (or maximum recorded): 3.43 doublings day^{-1}

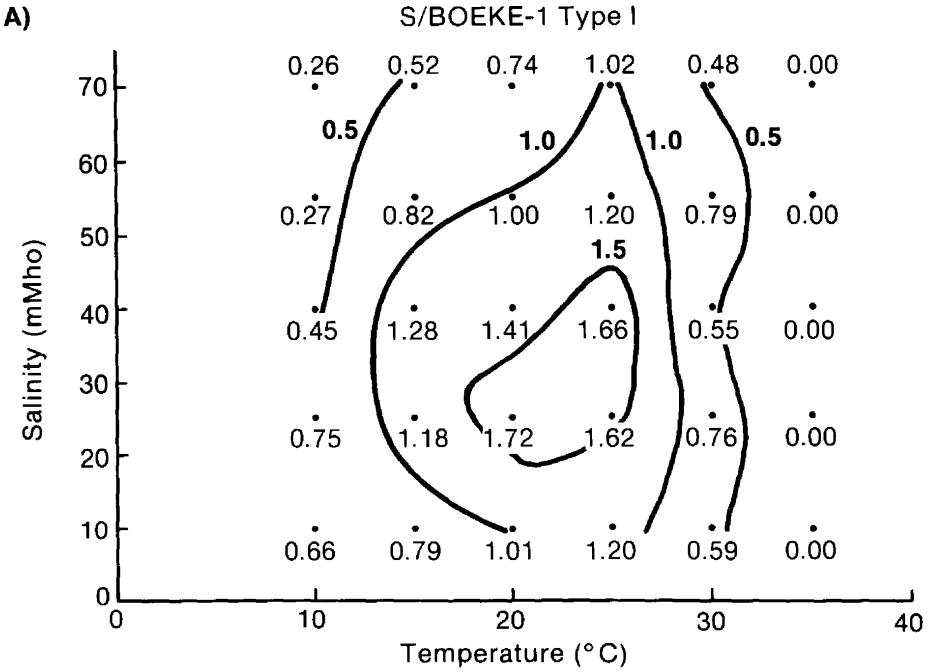
Culture conditions:

Vitamins required:	not determined
Available nitrogen sources:	urea, ammonium
Suitable media:	Type II/25
Nutritional modes:	autotrophic

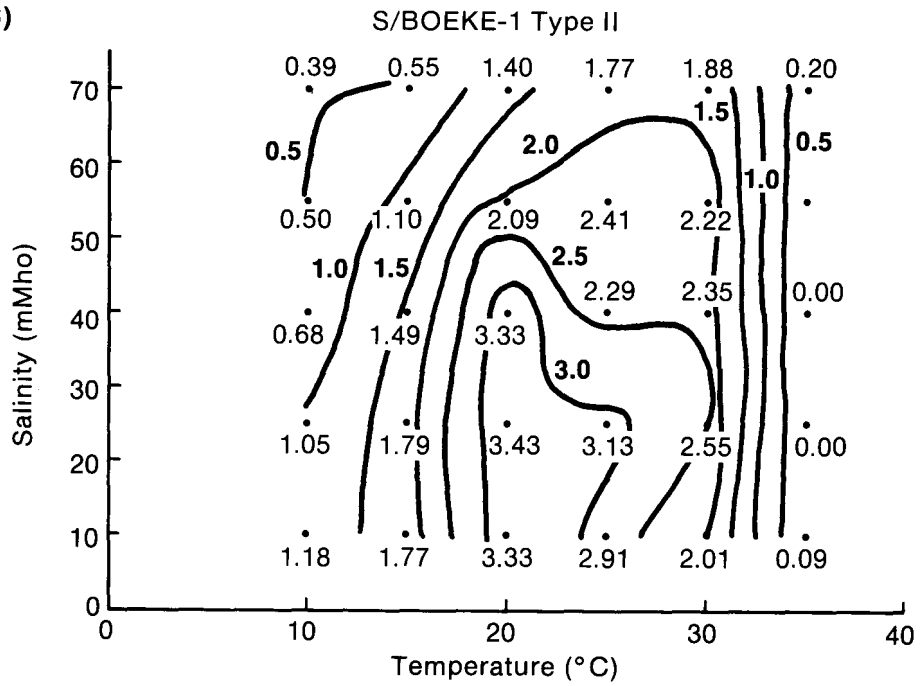
Temperature/salinity growth responses:

Exponential growth rate (doublings day^{-1}) in batch culture.

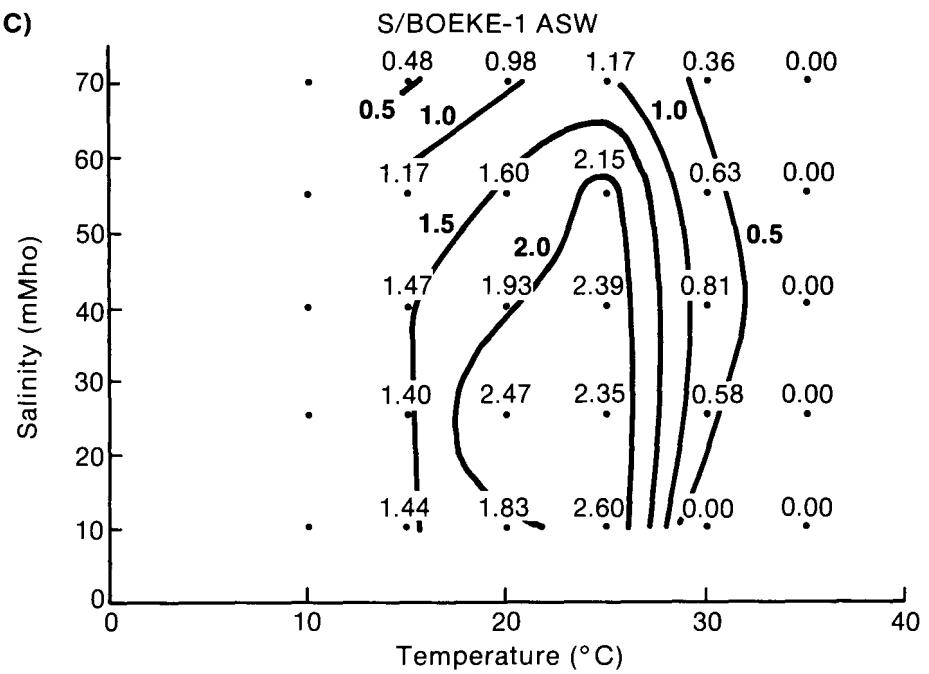
A = SERI Type I inland saline water;
B = SERI Type II inland saline water; and
C = artificial seawater.



B)


S/BOEKE-1 Type II
Salinity (mMho)
Temperature (°C)
0.39 0.55 1.40 1.77 1.88 0.20
0.5
2.0
1.5
1.0
0.5
0.50 1.10 2.09 2.41 2.22
1.0 1.5
2.5
2.29 2.35 0.00
0.68 1.49 3.33
3.0
1.05 1.79 3.43 3.13 2.55 0.00
1.18 1.77 3.33 2.91 2.01 0.09
0 10 20 30 40
0 10 20 30 40 50 60 70

C)


S/BOEKE-1 ASW
Salinity (mMho)
Temperature (°C)
0.48 0.98 1.17 0.36 0.00
0.5 1.0
1.0
1.17 1.60 2.15 0.63 0.00
1.5
2.0
0.5
1.47 1.93 2.39 0.81 0.00
1.40 2.47 2.35 0.58 0.00
1.44 1.83 2.60 0.00 0.00
0 10 20 30 40
0 10 20 30 40 50 60 70

Chemical composition:

Growth Conditions	% lipid	% protein	% carbohydrate	Ref.	Basis
C	23-29*				AFDW
C,N(N)	30.6*				AFDW
C,N(N, severe)	42.2*				AFDW
B	15.3‡			1	AFDW
B,N(N)	20.7‡			1	AFDW

*Analyses of fresh-frozen samples

‡Analyses of lyophilized samples

Lipid composition:

Growth Conditions	Fraction eluted by:					
	Hexane	Benzene	Chloroform	Acetone	Methanol	Ref.
B	0.7	7.7	10.2	51.4	29.9	1
B,N(N)	2.1	4.2	5.7	54.5	33.5	1

Physiological notes:

1. Shows improved growth in high-bicarbonate media.
2. Has a high nitrogen requirement [Q_{0N} is about 0.08 mol N (mol C)$^{-1}$].
3. pH optima are:
   (1) 7.5 or less in seawater
   (2) 8.0-8.5 in SERI Type II 25 mmho cm^{-1}
   (3) approximately 9.0 in artificial Piceance Creek Water.

Life cycle:

Only asexual reproduction through vegetative cell division has been observed in this strain. Statocyst formation has not been observed in laboratory cultures.

Outdoor culture history:

Attempts to cultivate outdoors at Vacaville, California, led to the rapid development of contaminants and predators. (2)

Literature cited:

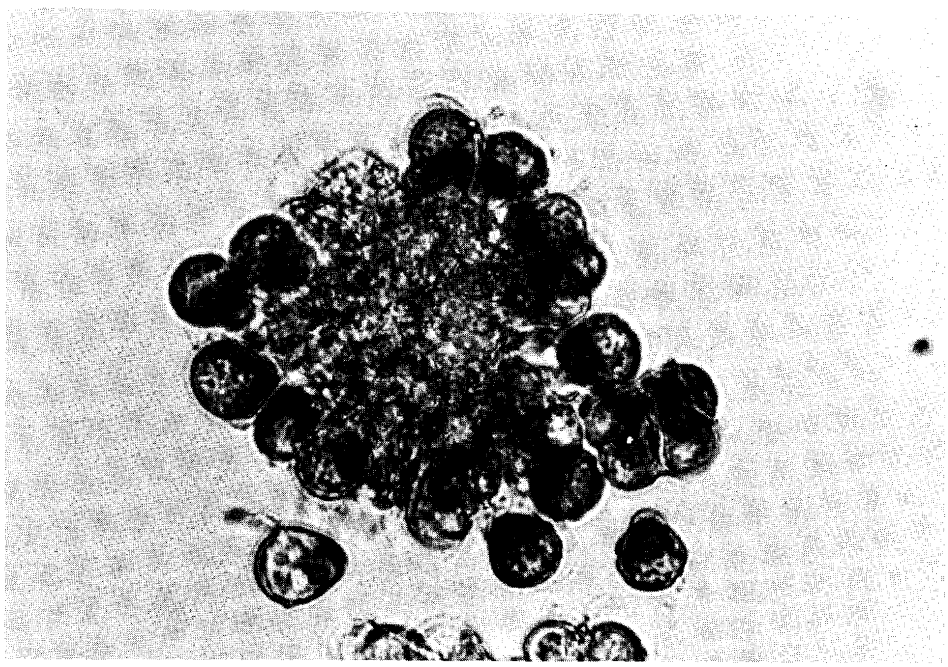
1. Benemann, J. Unpublished data.

2. Weissman, J. Unpublished data.

Botryococcus braunii **Kutz**

Strain: S/BOTRY-1 (UTEX #572)

Taxonomy: **Division:** Chlorophyta
Class: Chlorophyceae
Order: Chlorococcales
Family: Dictyosphaeriaceae



Colony of *Botryococcus braunii* S/BOTRY-1 (Scale: 1 cm = 10 μm)

Source: Univ. of Texas culture collection

Size: Individual cells = 11-12 μm x 8-10 μm

Growth form: colonial

Growth rate at optimum (or maximum recorded): 1.80 doublings day^{-1} (2)

Culture conditions:

Vitamins required: none

Available nitrogen sources: nitrate (best), ammonium (1)

Suitable media: modified Chu medium, *Botryococcus* medium

Nutritional modes: autotrophic, heterotrophic

Temperature range: not determined

optimum: not determined

Salinity range: not determined

optimum: not determined

Chemical composition:

Growth Conditions	% lipid	% protein	% carbohydrate	Ref.	Basis
B	44.5	22.0	14.1	2	AFDW
B, N(N)	54.2	20.6	14.3	2	AFDW
B, Saline	46.3	15.0	13.3	2	AFDW

Lipid composition:

Growth Conditions	Fraction eluted by:					Ref.
	Hexane	Benzene	Chloroform	Acetone	Methanol	
B	4.6	51.4	4.5	30.0	9.4	2
B, N(N)	14.9	52.7	3.4	21.6	7.4	2
B, Saline	5.2	46.0	28.5	9.3	9.7	2

Fuel production options:

Option	Methane MJ/kg	Ester MJ/kg	Hydro-carbon MJ/kg	Ethanol MJ/kg	Total Recovered MJ/kg	Fraction Utilized	Liquid Fuel MJ/kg
1	21.4	0	0	0	21.4	0.755	0
2	21.4	0	0	0	21.4	0.755	0
3	4.6	1.9	10.1	0	16.5	0.583	11.9
4	19.8	0	0	1.4	21.2	0.749	1.4
5	3.0	1.9	10.1	1.4	16.3	0.577	13.3

Total energy content: 28.3 MJ/kg dry weight

Physiological notes:

1. Organic nutrients (e.g., glucose) increase hydrocarbon production in *Botryococcus*. (3)

2. Cells cultured in 0.5 M NaCl exhibit a decrease in their production of C-30 hydrocarbon. (2)

3. C-30 and C-31 hydrocarbons amount to 59% of the major aliphatic hydrocarbons under nitrogen limited conditions. (2)

Life cycle:

Reproduction by colony fragmentation and autospore formation.

Outdoor culture history:

Attempts to culture *Botryococcus* in open air conditions in France resulted in low hydrocarbon production (<10% of dry weight) and competition from invading *Scenedesmus* and *Chlorella* spp. (4)

Literature cited:

1. Chu, S.P. 1943. The influence of the mineral composition of the medium on the growth of planktonic algae. *J. Ecol.* 31:284-325.

2. Ben-Amotz, A., T.G. Tornabene & W.H. Thomas. 1985. Chemical profiles of selected species of microalgae with emphasis on lipids. *J. Phycol.* 21: 72-81.

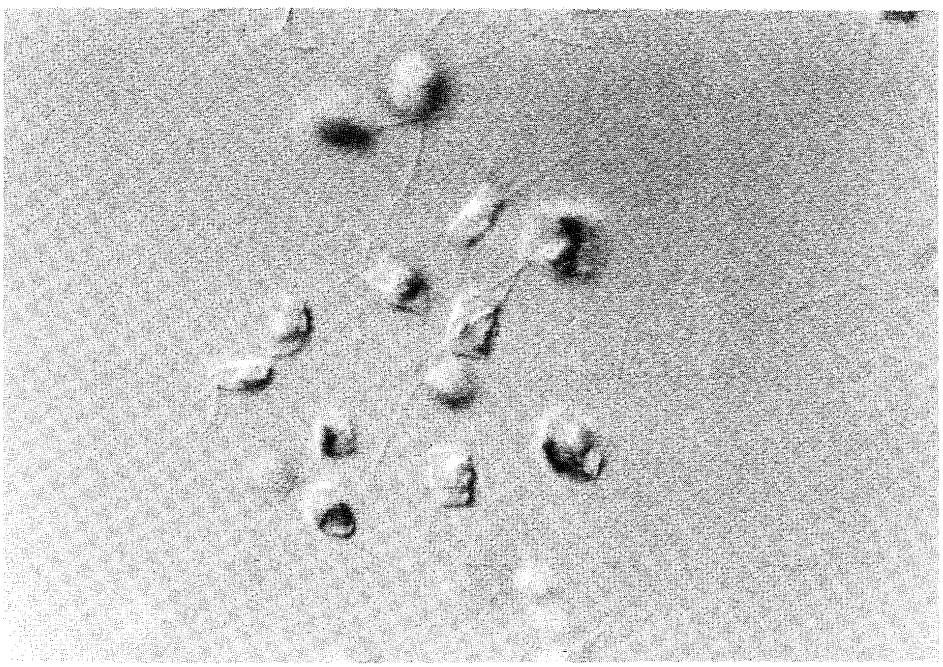
3. Tornabene, T.G. 1984. Chemical profile of microalgae with emphasis on lipid. In: *Aquatic Species Program Review: Proceedings of the April 1984 Principal Investigators Meetings.* Solar Energy Research Institute Publication SERI/CP-231-2341. pp. 64-78.

4. Destordeur, M., M.E. Rossi & C. Sironval. 1982. Culture de l'algue *Botrycoccus braunii* a l'echelle pilote. In: *Energy from Biomass.* Palz, W. & G. Grassi, (eds.). D. Reidel Publishing Co. pp. 153-165.

Chaetoceros gracilis Schutt

Strain: S/CHAET-1

Taxonomy: **Division:** Chrysophyta
Class: Bacillariophyceae
Order: Centrales
Family: Chaetoceraceae



Cells of *Chaetoceros gracilis* S/CHAET-1 (Scale: 1 cm = 10 µm)

Source: R. York, Hawaii Institute of Marine Biology, Kaneohe, Hawaii, USA

Size: 5-7 µm x 4 µm (setae 30-37 µm)

Growth form: unicells, chains

Growth rate at optimum (or maximum recorded): 4.3 doublings day^{-1} (1)

Culture conditions:

Vitamins required:	none (2)
Available nitrogen sources:	ammonium, nitrate, urea
Suitable media:	GPM
Nutritional modes:	photoautotrophic
Temperature range:	not determined
optimum:	28°-32°C (3)
Salinity range:	15-35 g TDS L^{-1}
optimum:	not determined

Chemical composition:

Growth Conditions	% lipid	% protein	% carbohydrate	Ref.	Basis
MC	20.5	48.6		4	DW
B	13			8	C
B	58			8	C

Physiological notes:

1. Populations crash rapidly (<12 h) in mass culture; crashes can be prevented by the addition of EDTA. (3)

2. Grows over a pH range from 7 to 9, with an optimum between 7 and 8. (1)

3. Growth as a function of total inorganic carbon content of the medium shows a half-saturation constant of less than 3 µM. (1)

4. Tolerates high oxygen concentrations. The growth rate at 500% O_2 saturation relative to air is reduced by 15%-25% from that observed at O_2 equilibrium. (1)

Life cycle:

Vegetative cell division is the ordinary method of reproduction. Sexual reproduction is oogamous, resulting in the formation of auxospores (zygotes). *Chaetoceros* can also form resting spores during conditions unfavorable for growth. (5)

Outdoor culture history:

1. *Chaetoceros* sp. was a component of an outdoor semicontinuous culture at Galway, Ireland. (6)

2. Also a component at Ghent, Belgium. (2)

3. Appeared in a continuous system that employed artificial upwelling, Seward, Alaska, USA. (7)

4. *C. gracilis* was grown in a penaeid hatchery as an exclusive food. (3)

Literature cited:

1. Weissman, J. Unpublished data.

2. dePauw, N., J. Verbonen & C. Claus. 1983. Large-scale microalgae production for nursery rearing of bivalve molluscs. *Aquacult. Engr.* 2: 27-47.

3. Simons, C.W. 1978. The culture of the diatom *Chaetoceros gracilis* and its use as a food for penaeid protozoean larvae. *Aquaculture* 14: 105-113.

4. Hirata, J. Unpublished data.

5. Drebes, G. 1977. Sexuality. In: *The Biology of Diatoms.* Werner, D. (ed.). University of California Press. pp. 250-283.

6. Roden, C.M. & K.W. O'Mahony. 1984. Competition as a mechanism of adaptation to environmental stress in outdoor cultures of marine diatoms. *Mar. Ecol. Prog. Ser.* 16: 219-228.

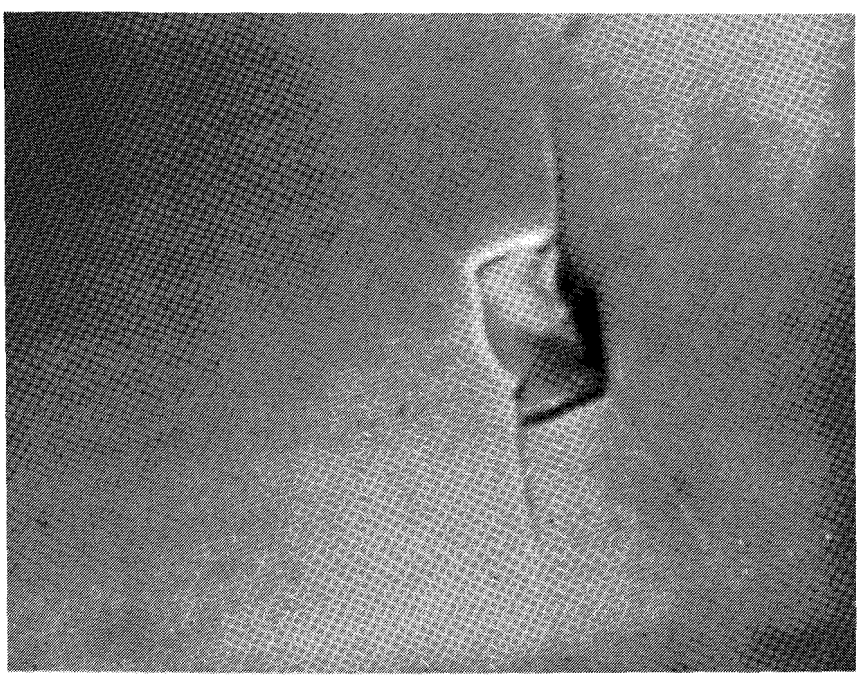
7. Paul, A.J., J.M. Paul & P.A. Shumaker. 1979. Artificial upwelling and phytoplankton production in Alaska. *Mar. Sci. Comm.* 5: 79-89.

8. Laws, E.A. 1985. Productivity optimization of saline microalgae grown in outdoor mass culture. In: *Aquatic Species Program Review: Proceedings of the March 1985 Principal Investigators' Meeting.* Solar Energy Research Institute Publication SERI/CP-231-2700. pp. 162-178.

Chaetoceros sp.

Strain: S/CHAET-2 (SS-14)

Taxonomy: **Division:** Chrysophyta
Class: Bacillariophyceae
Order: Centrales
Family: Chaetoceraceae



Cell of *Chaetoceros* sp. S/CHAET-2 (Scale 1 cm = 2.5 μm)

Collection site: Salton Sea, California, USA (W. Thomas) (1)

Date: August 10, 1984
Water temperature: 35.6°C

Size: 6 μm x 4 μm

Growth form: unicells, short chains

Growth rate at optimum (or maximum recorded): 4.3 doublings day^{-1} (3)

Culture conditions:

Vitamins required: not determined

Available nitrogen sources: nitrate, ammonium, urea

Suitable media: f/2, GPM, SERI Type II 25 mmho cm^{-1}, ASW

Nutritional modes: autotrophic

Temperature range: 20°-40°C (1)

optimum: 25°-35°C (1)

Salinity range: 10-40 g TDS L^{-1} (1)

optimum: 15 g TDS L^{-1} (1)

Chemical composition:

Growth Conditions	% lipid	% protein	% carbohydrate	Ref.	Basis
MC	23			3	AFDW
B, N(N)	22.2*	31.9	43.0	2	AFDW
	32.7‡				AFDW

*Analyses of lyophilized material

‡Analyses of fresh frozen material (same sample)

Fuel production options:

Option	Methane MJ/kg	Ester MJ/kg	Hydro-carbon MJ/kg	Ethanol MJ/kg	Total Recovered MJ/kg	Fraction Utilized	Liquid Fuel MJ/kg
1	16.6	0	---	0	16.6	0.702	0
2	16.6	0	---	0	16.6	0.702	0
3	9.4	6.9	---	0	16.3	0.691	6.9
4	11.5	0	---	4.2	15.7	0.666	4.2
5	4.7	6.9	---	4.2	15.8	0.669	11.1

Total energy content: 23.6 MJ/kg dry weight

Physiological notes:

1. pH range 6-9, optimum 7-8. (3)

2. Growth as a function of total inorganic carbon concentration shows a half-saturation constant of less than 3 μM. (3)

3. Not sensitive to inhibition of growth by high dissolved oxygen concentrations. The growth rate is reduced by only 10% when the oxygen concentration is raised from 100% to 500% of the air equilibrium value. (3)

Life cycle:

Vegetative cell division is the ordinary method of reproduction. Sexual reproduction results in the formation of auxospores. Resting spore formation has been observed in this strain.

Outdoor culture history:

This strain of *Chaetoceros* sp. was grown outdoors in 1.4 m^2 ponds at Vacaville, California, during August and September 1985 in artificial seawater with pH controlled at 7.5-8.0 by automated CO_2 additions. It produced an average of 25 g AFDW m^{-2} d^{-1} over 28 days. (3)

Literature cited:

1. Thomas, W.H., D.L.R. Seibert, M. Alden & P. Eldridge. 1985. Selection of desert saline microalgae for high yields at elevated temperatures and light intensities and in SERI standard artificial media. In: *Aquatic Species Program Review: Proceedings of the March 1985 Principal Investigators' Meeting.* Solar Energy Research Institute Publication SERI/CP-231-2700. pp. 5-27.

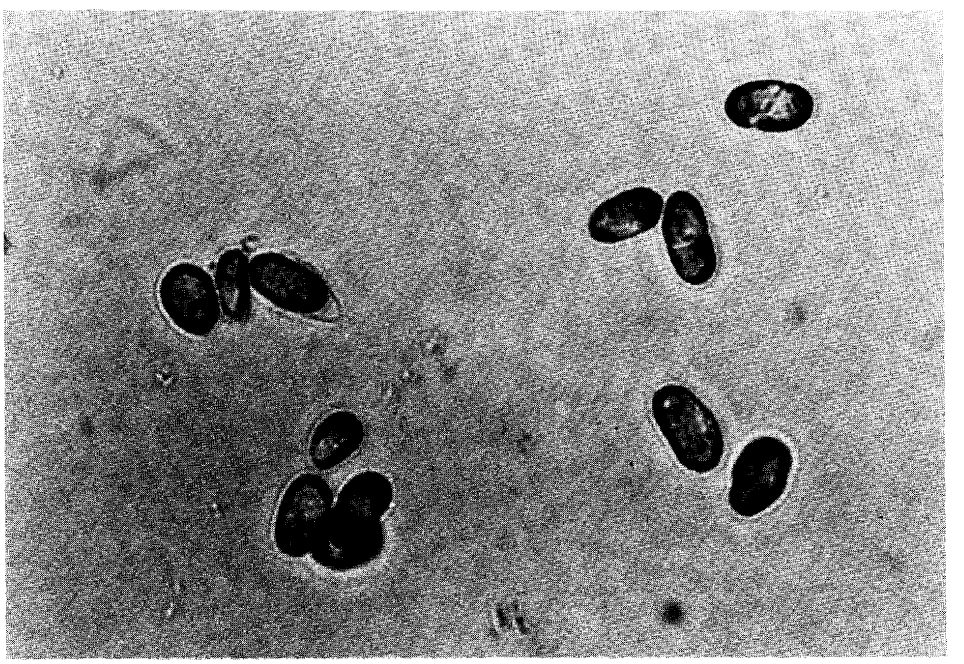
2. Benemann, J. Unpublished data.

3. Weismann, J. Unpublished data.

Chlorella sp.

Strain: S/CHLOR-1 (SO1)

Taxonomy: **Division:** Chlorophyta
Class: Chlorophyceae
Order: Chlorococcales
Family: Oocystaceae



Cells of *Chlorella* sp. S/CHLOR-1 (Scale: 1 cm = 10 μm)

Collection site: Construction ditch, Golden, Colorado, USA (S. Lien)

Date: June 3, 1980
Water temperature: 34°C
Salinity: Fresh water
pH: 7.3

Size: 6-10 μm exponential growth, 10-20 μm stressed (1)

Growth form: unicells

Growth rate at optimum (or maximum recorded): 1.33 doublings day^{-1}

Culture conditions:

Vitamins required: none

Available nitrogen sources: nitrate, ammonium, urea

Suitable media: Bolds Basal

Nutritional modes: photoautotrophic

Temperature range: 15°-39°C (1)

optimum: 35°C (1)

Salinity range: 0-18 g TDS L^{-1} (1)

optimum: 2-3 g TDS L^{-1} (1)

Chemical composition:

Growth Conditions	% lipid	% protein	% carbohydrate	Ref.	Basis
1 week old agar plate	13-20	42-51	----	1	DW
6 week old agar plate	39	14-33	----	1	DW
B	10	38-42	----	1	DW
B, N(N)	34-48	19-31	----	1	DW

Fuel production options:

Option	Methane MJ/kg	Ester MJ/kg	Hydro-carbon MJ/kg	Ethanol MJ/kg	Total Recovered MJ/kg	Fraction Utilized	Liquid Fuel MJ/kg
1	22.4	0	0	0	22.4	0.740	0
2	22.4	0	0	0	22.4	0.740	0
3	6.5	0	0	0	6.4	0.214	0
4	20.6	0	0	1.6	22.2	0.733	1.6
5	4.7	0	0	1.6	6.3	0.208	1.6

Total energy content: 30.3 MJ/kg dry weight

Physiological notes:

1. Ash = 4%-8% of dry weight. (1)

2. A salinity increase in cultures from 0 o/oo to 6 o/oo reduces lipid yield by 41%. (2)

3. 97% of total detectable nitrate reductase activity is lost within 6 hours of nitrogen depletion. (3)

Life cycle:

Reproduction is exclusively asexual. Each mature cell divides producing four or eight autospores which are freed by rupture of the parental cell wall.

Outdoor culture history:

1. Cultivation (autotrophic) costs (medium, water, and electricity) of *Chlorella* in Japan in 1980 were $1.517/kg. (4)

2. *Chlorella* spp. dominated an outdoor mass culture system utilized in recycling livestock wastes in Florida. Net productivity on a crop yield basis reached 30 g $m^{-2}d^{-1}$. (5)

3. Production of *Chlorella* in Asia exceeds 1000 kg of dried microalgae per month with average yield of 25-30 g $m^{-2}d^{-1}$. (4)

4. Fungal parasites were a problem in outdoor mass cultivation of *Chlorella* in Thailand. (6)

Literature cited:

1. Lien, S. 1984. Unpublished data.

2. Lien, S. & K.G. Spencer. 1983. Algal oil production and lipid metabolism. In: *Aquatic Species Program Review: Proceedings of the March 1983 Principal Investigators Meetings.* Solar Energy Research Institute Publication SERI/CP-231-1946. pp. 3-19.

3. Lien, S. & K.G. Spencer. 1984. Physiology of oil producing microalgae in response to stress conditions. In: *Aquatic Species Program Review: Proceedings of the April 1984 Principal Investigators Meetings.* Solar Energy Research Institute Publication SERI/CP-231-2341. pp. 79-108.

4. Kawaguchi, K. 1980. Microalgae production systems in Asia. In: *Algae Biomass.* Shelef, G. & C.J. Soeder (eds.). Elsevier/North-Holland Biomedical Press. pp. 25-33.

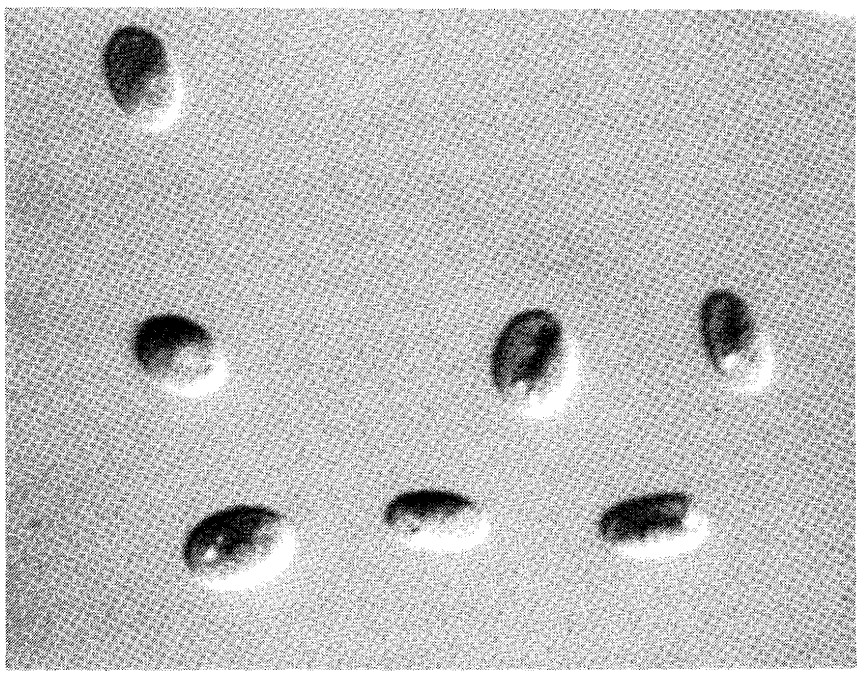
5. Lincoln, A.P. & D.T. Hill. 1980. An integrated microalgae system. In: *Algae Biomass.* Shelef, G. & C.J. Soeder (eds.). Elsevier/North-Holland Biomedical Press. pp. 229-244.

6. Sinchumpasak, O. 1980. Microalgal biomass production in Thailand. In: *Algae Biomass.* Shelef, G. & C.J. Soeder (eds.). Elsevier/North-Holland Biomedical Press. pp. 115-121.

Chlorella ellipsoidea

Strain: S/CHLOR-2 (BL-6)

Taxonomy: **Division:** Chlorophyta
Class: Chlorophyceae
Order: Chlorococcales
Family: Oocystaceae



Cells of *Chlorella ellipsoidea* S/CHLOR-2 (Scale: 1 cm = 3.8 μm)

Collection site: Black Lake, California, USA (W. Thomas) (1)

Date: May 16, 1984
Water temperature: 15°C
Salinity: 15.5 g TDS L^{-1}

Size: 6-8 μm x 4 μm

Growth form: unicells

Growth rate at optimum (or maximum recorded): 5.3 doublings day^{-1}

Culture conditions:

Vitamins required:	not determined
Available nitrogen sources:	nitrate, ammonium
Suitable media:	SERI Type II, 10 or 25 mmho cm^{-1}
Nutritional modes:	autotrophic
Temperature range:	20°-35°C (1)
optimum:	approx. 30°C (1)
Salinity range:	10->40 g TDS L^{-1} (1)
optimum:	approx. 20 g TDS L^{-1} (1)

Chemical composition:

Growth Conditions	% lipid	% protein	% carbohydrate	Ref.	Basis
B	10.9	34.2	20.5	2	DW
B,N(N)	8.9	26.1	26.3	2	DW
B	14.2*	32.0	30.7	3	AFDW
	15.9‡				AFDW
B,N(N)	14.8*	29.7	43.2	3	AFDW
	20.9‡				AFDW
B,N(N,severe)	12.2*	10.2	50.2	3	AFDW
	30.1‡				AFDW

* Lyophilized material

‡ Same material as *, but extracted as fresh-frozen material.

Fuel production options:

Option	Methane MJ/kg	Ester MJ/kg	Hydro-carbon MJ/kg	Ethanol MJ/kg	Total Recovered MJ/kg	Fraction Utilized	Liquid Fuel MJ/kg
1	17.4	0	---	0	17.4	0.729	0
2	17.4	0	---	0	17.4	0.729	0
3	7.0	9.4	---	0	16.4	0.688	9.5
4	10.8	0	---	4.9	15.7	0.659	4.92
5	1.5	9.4	---	4.9	15.8	0.663	14.3

Total energy content: 23.9 MJ/kg dry weight

Physiological notes:

1. Grows over a pH range of 6-10, with optimum performance between pH 6 and 7.5. (4)

2. Growth as a function of total inorganic carbon concentration shows a half saturation constant of less than 5 μM. (4)

3. This strain is sensitive to inhibition of growth by oxygen. The growth rate at 500% O_2 saturation is only 30% of that at O_2 equilibrium. (4)

Life cycle:

Reproduction is exclusively asexual. Each mature cell divides producing four or eight autospores that are freed by rupture of the parental cell wall.

Outdoor culture history:

This strain of *Chlorella ellipsoidea* was grown outdoors in 1.4 m^2 tanks at Vacaville, California, with pH controlled at 7.5-8.0 by CO_2 additions in SERI Type II (low salinity) medium. It produced an average of 15 g AFDW m^{-2} d^{-1} under these conditions when the oxygen tension in the system was reduced to 150%-200% saturation by sparging. Without sparging, the oxygen concentration rose to about 500% saturation, which led to failure of the culture. (4)

Literature cited:

1. Thomas, W.H., D.L.R. Seibert, M. Alden & P. Eldridge. 1985. Selection of desert saline microalgae for high yields at elevated temperatures and light intensities and in SERI standard artificial media. In: *Aquatic Species Program Review: Proceedings of the March 1985 Principal Investigators' Meeting.* Solar Energy Research Institute Publication SERI/CP-231-2700. pp. 5-27.

2. Tornabene, T.G. & J.R. Benemann. 1985. Chemical profiles on microalgae with emphasis on lipids. In: *Aquatic Species Program Review: Proceedings of the March 1985 Principal Investigators' Meeting.* Solar Energy Research Institute Publication SERI/CP-231-2700. pp. 83-99.

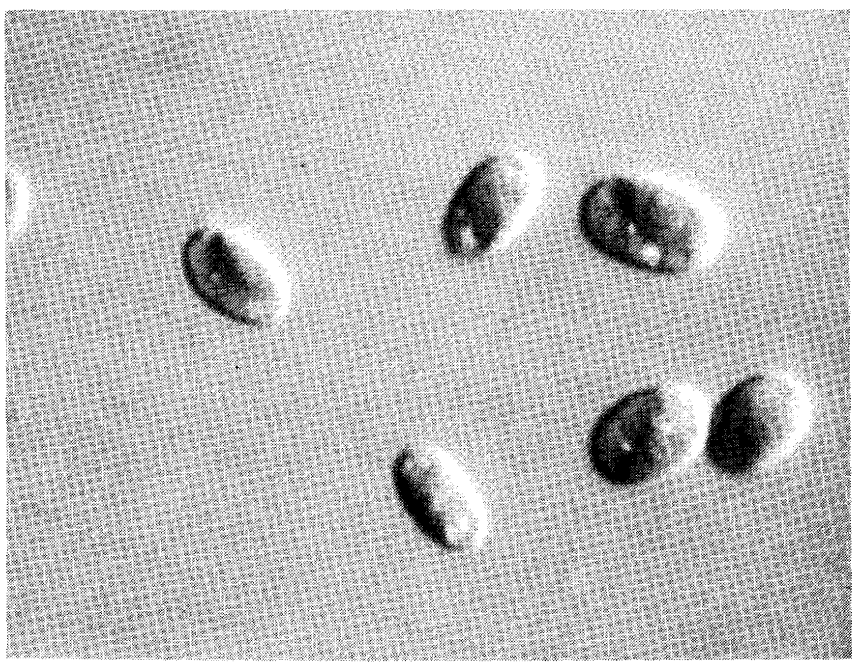
3. Benemann, J. Unpublished data.

4. Weissman, J. Unpublished data.

Chlorella sp.

Strain: S/CHLOR-3 (SC-2)

Taxonomy: **Division:** Chlorophyta
Class: Chlorophyceae
Order: Chlorococcales
Family: Oocystaceae



Cells of *Chlorella* sp. S/CHLOR-3 (Scale: 1 cm = 3.8 μm)

Collection site: Salt Creek, California, USA (W. Thomas) (1)

Date: July 30, 1984
Water temperature: 38°C
Salinity: 13.5 g TDS L^{-1}

Size: 6 μm x 4 μm ovals

Growth form: unicells

Growth rate at optimum (or maximum recorded): 1.88 doublings day^{-1} (1)

Culture conditions:

Vitamins required:	not determined
Available nitrogen sources:	nitrate, ammonium
Suitable media:	SERI Type II, 10 mmho cm^{-1}
Nutritional modes:	autotrophic
Temperature range:	20°->40°C (1)
optimum:	approx. 30°C (1)
Salinity range:	1-25 g TDS L^{-1} (1)
optimum:	approx. 10 g TDS L^{-1} (1)

Life cycle:

Reproduction is exclusively asexual. Each mature cell divides producing four or eight autospores which are freed by rupture of the parent cell wall.

Literature cited:

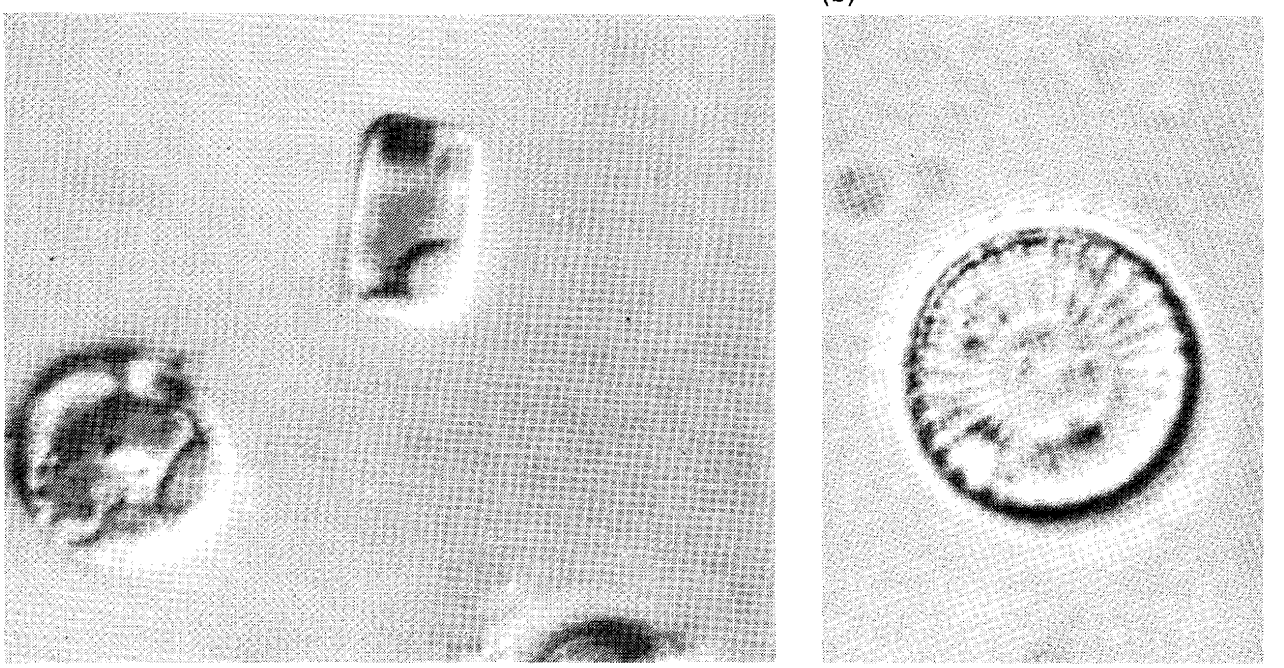
Thomas, W.H., D.L.R. Seibert, M. Alden & P. Eldridge. 1985. Selection of desert saline microalgae for high yields at elevated temperatures and light intensities and in SERI standard artificial media. In: *Aquatic Species Program Review: Proceedings of the March 1985 Principal Investigators' Meeting.* Solar Energy Research Institute Publication SERI/CP-231-2700. pp. 5-27.

Cyclotella sp.

Strain: S/CYCLO-1 (DI-35)

Taxonomy: **Division:** Chrysophyta
Class: Bacillariophyceae
Order: Centrales
Family: Coscinodiscaceae

(a) (b)



Cells of *Cyclotella* sp. S/CYCLO-1 (Scale: 1 cm = 3.8 µm)
(a) valve and girdle view, (b) valve view of frustule

Collection site: Dauphin Island, Gulf of Mexico (M. Tadros) (1)

Date: November 1983
Water temperature: 22°C
Salinity: 15 g TDS L^{-1}
pH: 7.5

Size: 13-15 µm

Growth form: unicells

Growth rate at optimum (or maximum recorded): 5.1 doublings day^{-1} (3)

Culture conditions:

Vitamins required: none

Available nitrogen sources: nitrate, urea

Suitable media: f/2, all SERI media at appropriate salinities

Nutritional modes: autotrophic

Temperature range: 25°->35°C (1)

optimum: 30°C (1)

Salinity range: 6->45 g TDS L^{-1} (1)

optimum: 6-15 g TDS L^{-1} (1)

Chemical composition:

Growth Conditions	% lipid	% protein	% carbohydrate	Ref.	Basis
B	13.2	12.2	37.5	1	AFDW
B,N(N)	42.1	16.4	10.2	1	AFDW
MC, high light	20			2	AFDW
MC, low light	30			2	AFDW
MC, N(Si)	42			2	AFDW

Lipid composition:

Growth Conditions	Fraction eluted by:					Ref.
	Hexane	Benzene	Chloroform	Acetone	Methanol	
B,N(N)	0.8	88.9	2.5	4.1	3.7	2
B,N(N)	1.3	63.2	7.9	17.5	10.0	2

Fuel production options:

Option	Methane MJ/kg	Ester MJ/kg	Hydro-carbon MJ/kg	Ethanol MJ/kg	Total Recovered MJ/kg	Fraction Utilized	Liquid Fuel MJ/kg
1	20.0	0	0	0	20.0	0.725	0
2	20.0	0	0	0	20.0	0.725	0
3	3.5	13.1	0	0	16.7	0.604	13.1
4	15.4	0	0	1.0	16.4	0.596	1.0
5	2.4	13.1	0	1.0	16.5	0.599	14.1

Total energy content: 27.6 MJ/kg dry weight

Physiological notes:

1. Grows over a pH range of 7-9, with optimum growth between 7 and 8. (3)

2. Not sensitive to inhibition of growth by high dissolved oxygen concentrations. The growth rate is reduced by only about 10% when the oxygen concentration is raised from 100% to 500% of the air equilibrium value. (3)

Life cycle:

Vegetative cell division is the ordinary method of reproduction. Sexual reproduction is oogamous, with sexual fusion resulting in the formation of an auxospore.

Outdoor culture history:

This strain was grown outdoors at Vacaville, California, during June, July, and August 1985 in 1.4 m^2 tanks. Growth was satisfactory in either SERI Type I or Type II medium, with pH controlled to 7.5-8.0. Production averaged 30 g m^{-2} d^{-1} over 33 days and 36 g m^{-2} d^{-1} over a 10-day period. (3)

Literature cited:

1. Tadros, M.G. 1985. *Screening and Characterizing Oleaginous Microalgal Species from the Southeastern United States.* Final Subcontract Report to the Solar Energy Research Institute. SERI/STR-231-2657.

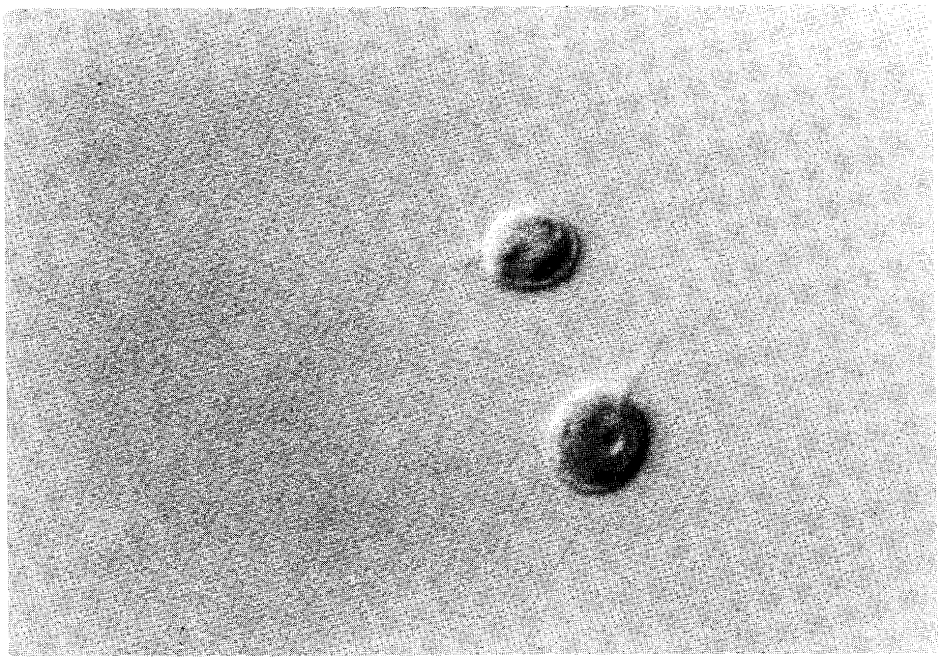
2. Benemann, J. Unpublished data.

3. Weismann, J. Unpublished data.

Isochrysis aff. *galbana* Green

Strain: S/ISOCH-1 (Tahitian T-ISO)

Taxonomy: **Division:** Chrysophyta
Class: Prymnesiophyceae
Order: Isochrysidales
Family: Isochrysidaceae



Cells of *Isochrysis* aff. *galbana* S/ISOCH-1 (Scale: 1 cm = 5 μm)

Source: R. York, Hawaii Institute of Marine Biology, Kaneohe, Hawaii, USA

Size: 7-4 μm x 4 μm

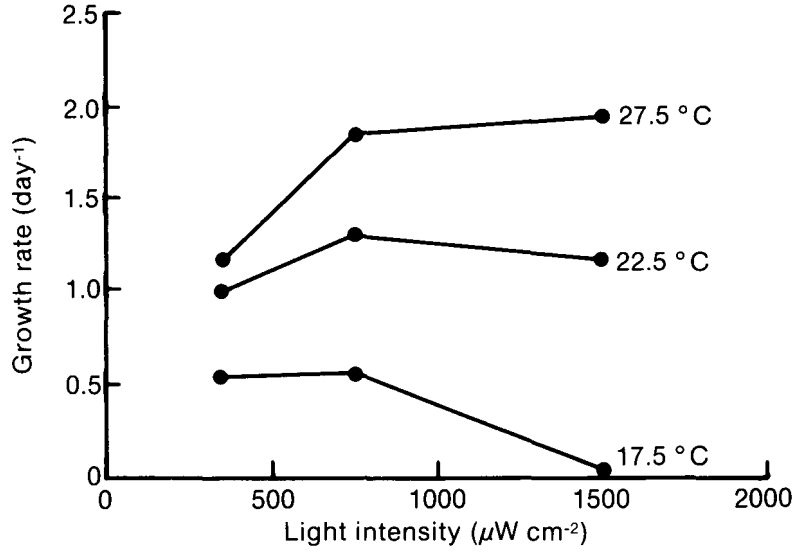
Growth form: flagellated unicells

Growth rate at optimum (or maximum recorded): 2.83 doublings day^{-1} (1)

Culture conditions:

Vitamins required:	not determined
Available nitrogen sources:	ammonium, nitrate
Suitable media:	ASW, f/2, GPM
Nutritional modes:	photoautrophic
Temperature range:	16°-34°C (1,2)
optimum:	28°C (2)
Salinity range:	5-60 g TDS L^{-1} (2)
optimum:	30-60 g TDS L^{-1} (2)

Light curve of growth:



after (1)

Chemical composition:

Growth Conditions	% lipid	% protein	% carbohydrate	Ref.	Basis
SC?	7.1	37.0	11.2	5	AFDW
SC?, N(N)	26.0	23.3	20.5	5	AFDW
SC*	20	21	14	3	AFDW
SC, N(N)*	19	12	25	3	AFDW

Lipid composition:

Growth Conditions	Fraction eluted by:					
	Hexane	Benzene	Chloroform	Acetone	Methanol	Ref.
SC? 1.4	27.4	32.1	26.3	12.6	5	
SC?, N(N)	2.2	28.4	18.0	26.0	25.3	5
SC*	1.5	15.2	13.5	31.6	38.2	3
SC, N(N)*	2.5	35.6	12.7	28.0	21.2	3

Fuel production options:

Option	Methane MJ/kg	Ester MJ/kg	Hydro-carbon MJ/kg	Ethanol MJ/kg	Total Recovered MJ/kg	Fraction Utilized	Liquid Fuel MJ/kg
1	13.7	0	0	0	13.7	0.723	0
2	13.7	0	0	0	13.7	0.723	0
3	5.7	3.7	1.9	0	11.3	0.595	5.6
4	11.4	0	0	2.0	13.5	0.710	2.0
5	3.4	3.7	1.9	2.0	11.0	0.582	7.6

Total energy content: 18.9 MJ/kg dry weight

Physiological notes:

1. A high proportion of the dry weight of *Isochrysis* sp. (~45%) was not extracted as protein, lipid, or carbohydrate. (1)

2. Tolerates pH from 5.5-9.0, with optimum at 6.0. (2)

3. Displays significant physiological differences from *I. galbana*. (3)

Life cycle:

Knowledge of the life cycle of this genus is very fragmentary. It probably has a sexual phase, but it has not been observed.

Outdoor culture history:

I. aff. *galbana* (T-ISO) has been grown outdoors in continuous culture as feed for bivalve molluscs. (4)

Literature cited:

1. Ewart, J.W. & G.D. Pruder. 1981. Comparative growth of *Isochrysis galbana* Parke and *Isochrysis* aff. *galbana*, clone T-ISO at four temperatures and three light intensities. *J. World Maricul. Soc.* 12:333-339.

2. Richmond, A. 1984. Development of outdoor system for production of lipid rich halotolerant microalgae. In: *Aquatic Species Program Review: Proceedings of the April 1984 Principal Investigators Meetings.* Solar Energy Research Institute Publication SERI/CP-231-2341. pp. 195-205. (Data presented in this publication are for a similar strain isolated in Israel, which is also available from the curator on request.)

3. Ben-Amotz, A. 1984. Development of outdoor raceway capable of yielding halotolerant microalgae, identification of oil-rich strains. In: *Aquatic Species Program Review: Proceedings of the April 1984 Principal Investigators Meetings.* Solar Energy Research Institute Publication SERI/CP-231-2341. pp. 186-194.

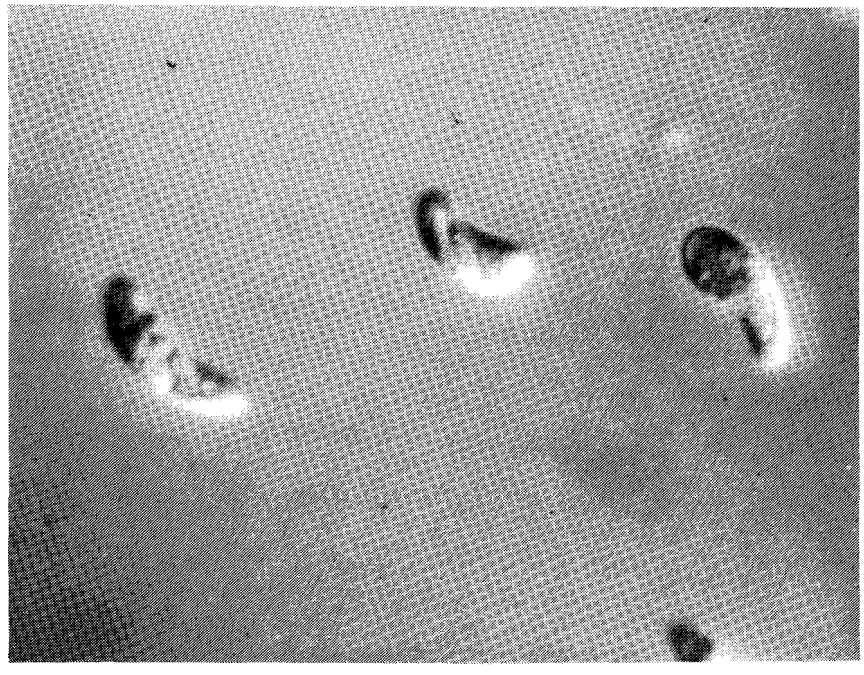
4. Goldstein, B.B. & O.A. Roels. 1980. The effect of feed density on the growth of juvenile *Mercenaria campechiensis*, the southern hard clam. In: *Proceedings of the Eleventh Annual Meeting, World Mariculture Society.* Avault, J.W., Jr. pp. 192-201.

5. Ben-Amotz, A., T.G. Tornabene & W.H. Thomas. 1985. Chemical profiles of selected species of microalgae with emphasis on lipids. *J. Phycol.* 21: 72-81.

Monoraphidium sp.

Strain: S/MONOR-1

Taxonomy: **Division:** Chlorophyta
Class: Chlorophyceae
Order: Chlorococcales
Family: Oocystaceae



Cells of *Monoraphidium* sp. S/MONOR-1 (Scale: 1 cm = 2.5 μm)

Collection site: Temporary pond near Stoner in southwestern Colorado, USA (W. Barclay)

Date: August 1984
Water temperature: 29°C
Salinity: 20 mmho cm^{-1} conductivity
pH: 9.5

Size: 6 μm x 2 μm

Growth form: unicells

Growth rate at optimum (or maximum recorded): 3.1 doublings day^{-1}

Culture conditions:

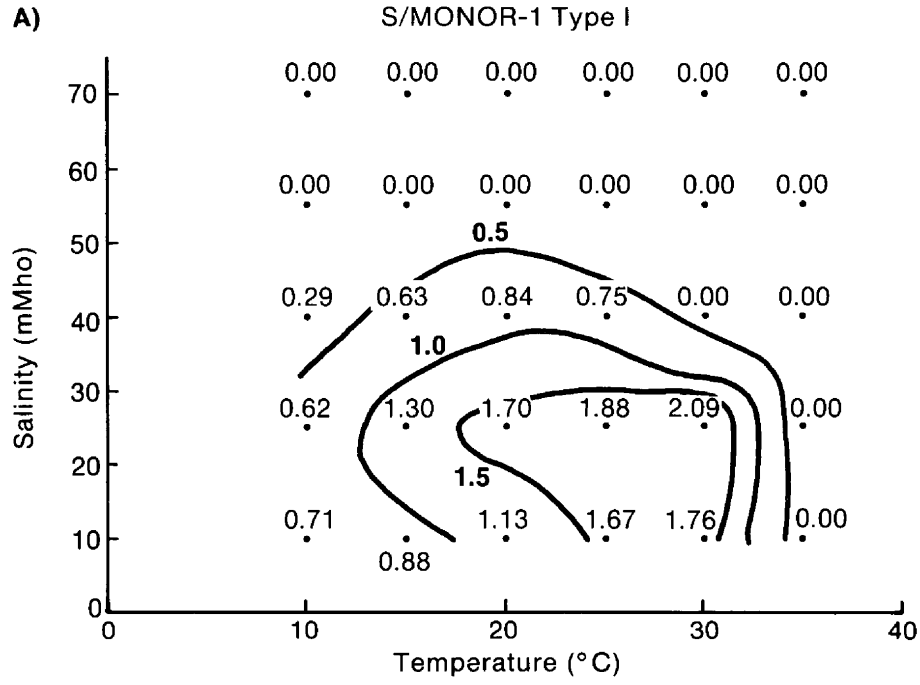
Vitamins required:	not determined
Available nitrogen sources:	urea, nitrate
Suitable media:	Type II/25
Nutritional modes:	photoautotrophic

Temperature/salinity growth responses:

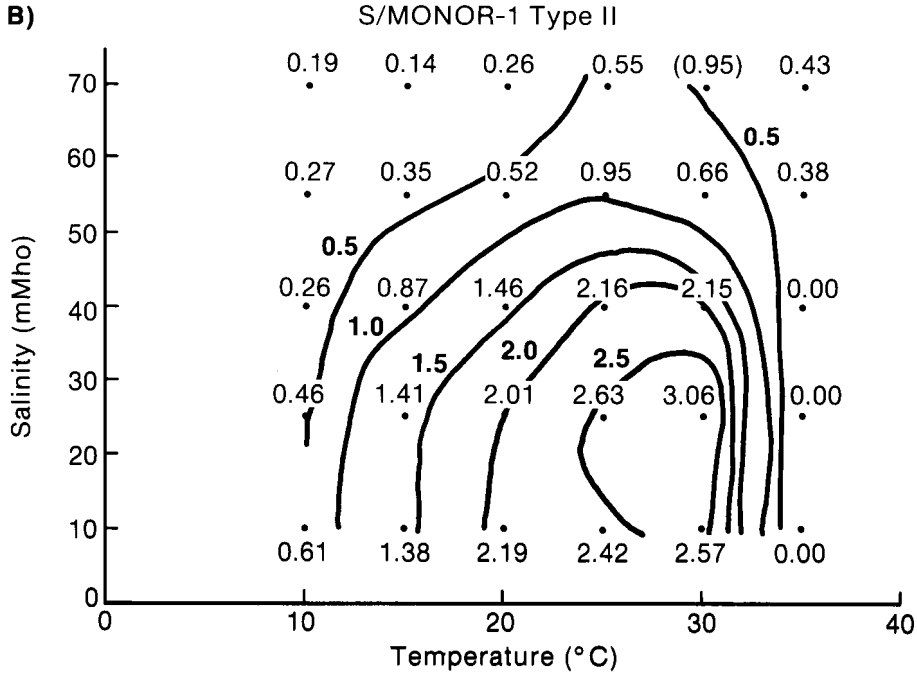
Exponential growth rate (doublings day^{-1}) in semicontinuous culture.

A = SERI Type I inland saline water; and

B = SERI Type II inland saline water.



B)



Chemical composition:

Growth Conditions	% lipid	% protein	% carbohydrate	Ref.	Basis
B	20.8		38.5	1	AFDW
B,N(N)	17.9		25.5	1	AFDW
B,N(N,severe)	25.3		27.0	1	AFDW
B	23.4				AFDW
B,N(N)	24.4				AFDW
B,N(N,severe)	29.4				AFDW

Fuel production options:

Option	Methane MJ/kg	Ester MJ/kg	Hydro-carbon MJ/kg	Ethanol MJ/kg	Total Recovered MJ/kg	Fraction Utilized	Liquid Fuel MJ/kg
1	17.0	0	0	0	17.0	0.700	0
2	17.0	0	0	0	14.5	0.597	0
3	6.6	7.9	0	0	14.5	0.600	7.9
4	11.5	0	0	2.7	14.1	0.583	2.7
5	3.7	7.9	0	2.7	14.2	0.586	10.5

Total energy content: 24.21 MJ/kg dry weight

Physiological notes:

Will grow in freshwater culture media.

Life cycle:

Reproduces asexually by the formation of auxospores.

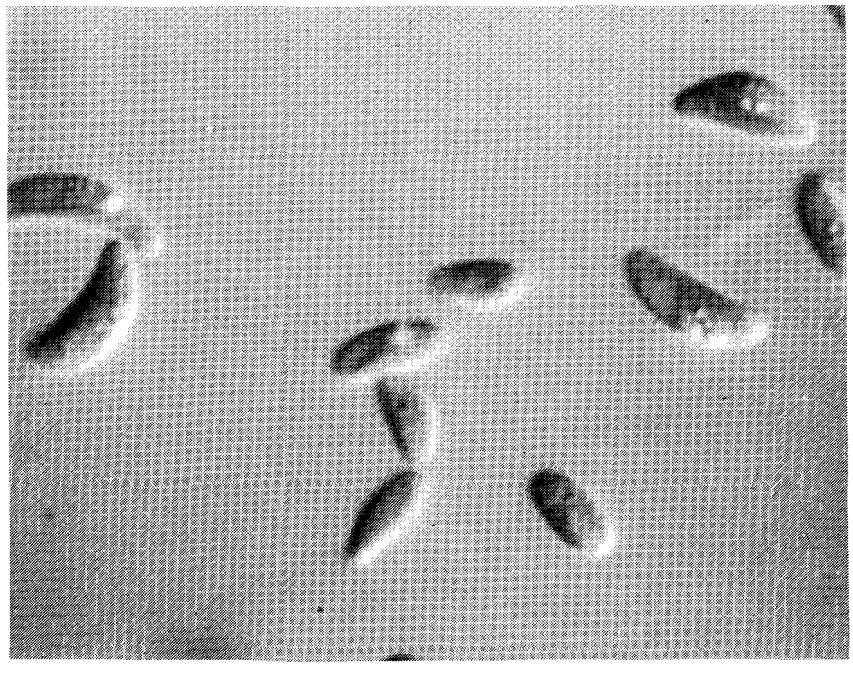
Literature cited:

Benemann, J. Unpublished data.

Monoraphidium sp.

Strain: S/MONOR-2

Taxonomy: **Division:** Chlorophyta
Class: Chlorophyceae
Order: Chlorococcales
Family: Oocystaceae



Cells of *Monoraphidium* sp. S/MONOR-2 (Scale: 1 cm = 2.5 μm)

Collection site: Temporary pond near Ridgeway, Utah, USA (W. Barclay)

Date: July 26, 1984
Water temperature: 29°C
Salinity: 25 mmho cm^{-1} conductivity
pH: 9.2

Size: 6 μm x 2 μm

Growth form: unicells

Growth rate at optimum (or maximum recorded): 5.8 doublings day^{-1} (1)

Culture conditions:

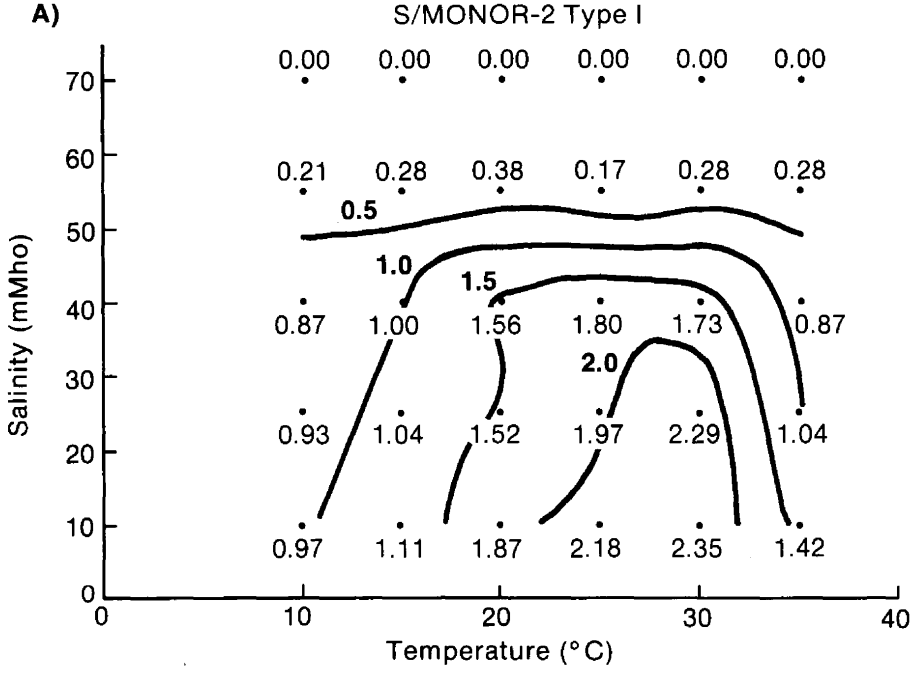
Vitamins required:	not determined
Available nitrogen sources:	urea, nitrate
Suitable media:	Type I/10
Nutritional modes:	photoautotrophic

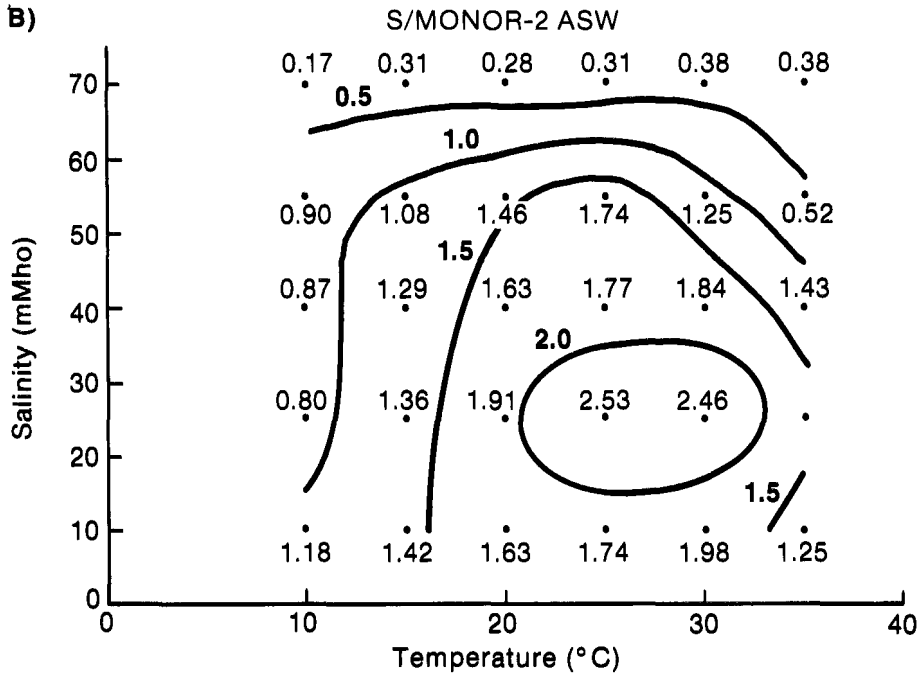
Temperature/salinity growth responses:

Exponential growth rate (doublings day^{-1}) in semicontinuous culture.

A = SERI Type I saline water; and

B = artificial seawater.





Physiological notes:

1. Grows over a pH range of 7-10, with optimum performance at about 9. (1)
2. Growth as a function of total inorganic carbon content shows a half-saturation constant of less than 3 μM. (1)
3. Reasonably insensitive to inhibition of growth by dissolved oxygen. The growth rate at 500% O_2 saturation relative to air is reduced by 25% over that observed at O_2 equilibrium. (1)
4. Will grow in freshwater culture media.

Life cycle:

Reproduces asexually by the formation of auxospores.

Outdoor culture history:

S/MONOR-2 was cultured outdoors at Vacaville, California, during September 1985 in a low salinity Type I medium, and showed a production rate of 15-20 g m^{-2} d^{-1}. (1)

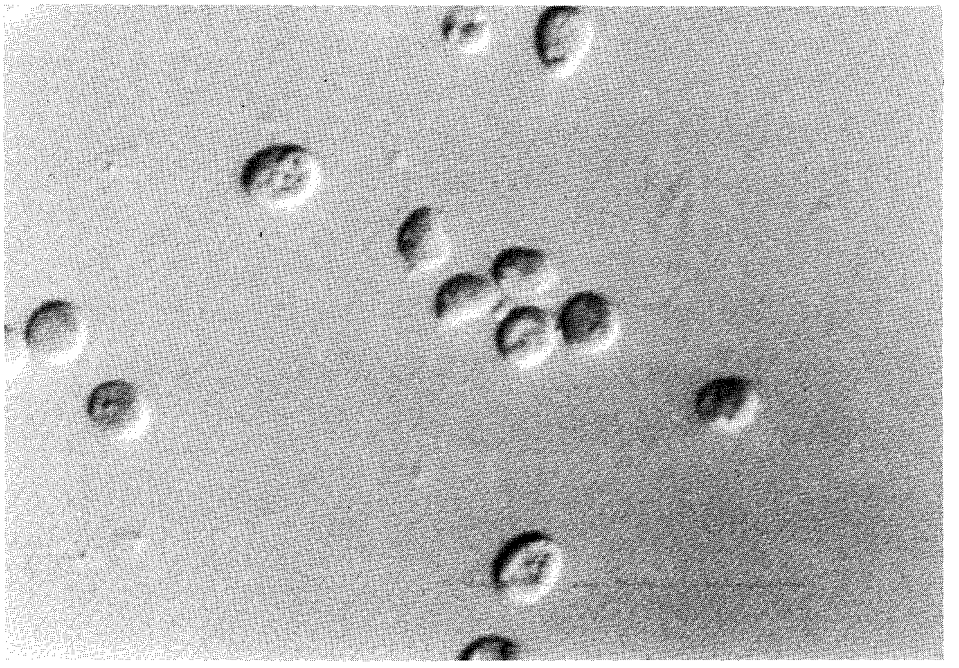
Literature cited:

Weissman, J. Unpublished data.

Nannochloropsis salina Hibberd

Strain: S/NANNO-1 (GBSTICHO)

Taxonomy: **Division:** Chrysophyta (1); Eustigmatophyta (2)
Class: Eustigmatophyceae
Order: Eustigmatales
Family: Monodopsidaceae



Cells of *Nannochloropsis salina* S/NANNO-1 (Scale: 1 cm = 5 μm)

Collection site: Great South Bay, Long Island, New York, USA (J. Ryther)

Date: 1952

Size: 2.5-5 μm x 1.5-1.7 μm

Growth form: unicells

Growth rate at optimum (or maximum recorded): 1.05 doublings day^{-1}

Culture conditions:

Vitamins required: not determined

Available nitrogen sources: ammonium, urea, nitrate

Suitable media: f/2

Nutritional modes: photoautotrophic

Temperature range: 17°-32°C (3)

optimum: 28°C (3)

Salinity range: 6-60 g TDS L^{-1} (3)

optimum: 30 g TDS L^{-1} (3)

Chemical composition:

Growth Conditions	% lipid	% protein	% carbohydrate	Ref.	Basis
B	28.6	55.8	15.6	4	AFDW
B, N(N)	59.8	24.3	15.9	4	AFDW

Lipid composition:

Growth Conditions	Fraction eluted by:					Ref.
	Hexane	Benzene	Chloroform	Acetone	Methanol	
B	2.5	12.4	25.4	28.7	31.0	5
B, N(N)	4.0	40.2	35.5	16.0	4.0	5

Fuel production options:

Option	Methane MJ/kg	Ester MJ/kg	Hydro-carbon MJ/kg	Ethanol MJ/kg	Total Recovered MJ/kg	Fraction Utilized	Liquid Fuel MJ/kg
1	23.8	0	0	0	23.8	0.753	0
2	23.8	0	0	0	23.8	0.753	0
3	5.3	8.9	6.5	0	20.7	0.656	15.4
4	22.0	0	0	1.6	23.6	0.747	1.6
5	3.5	8.9	6.5	1.6	20.5	0.650	17.0

Total energy content: 31.6 MJ/kg dry weight

Physiological notes:

1. pH range 5.0-10.5, optimum = 9.0. (3)
2. Lipid content is influenced by medium (natural or artificial) as well as pH and nitrogen source. Greatest lipid production on ammonium in natural seawater (pH 7.5-8.0). (4)

Life cycle:

Knowledge of the life cycle of this genus is very fragmentary. Only asexual reproduction has been observed.

Outdoor culture history:

Poor competitor at low temperatures in mass culture. (3)

Literature cited:

1. Bold, H.C. & M.J. Wynne. 1978. *Introduction to the Algae.* Prentice-Hall, New Jersey. 706 pp.

2. Hibberd, D.J. 1981. Notes on the taxonomy and nomenclature of the algal classes Eustigmatophyceae and Tribophyceae (synony Xanthophyceae). *Bot. J. Limn. Soc.* 82:93-119.

3. Richmond, A. 1984. Development of outdoor system for production of lipid-rich halotolerant microalgae. In: *Aquatic Species Program Review: Proceedings of the April 1984 Principal Investigators Meetings.* Solar Energy Research Institute Publication SERI/CP-231-2341. pp. 184-224.

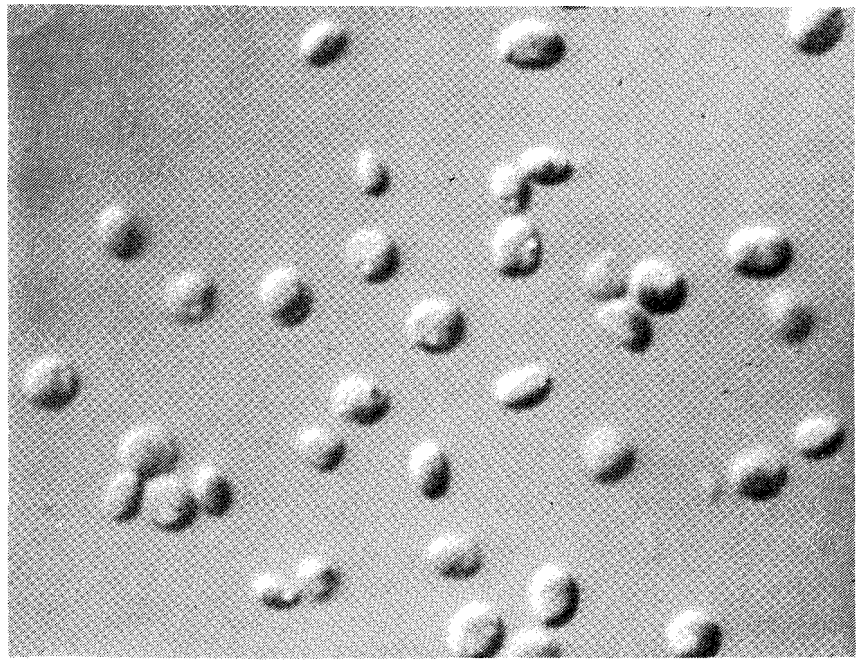
4. Tornabene, T.G. 1984. Chemical profile of microalgae with emphasis on lipids. In: *Aquatic Species Program Review: Proceedings of the April 1984 Principal Investigators Meetings.* Solar Energy Research Institute Publication SERI/CP-231-2341. pp. 64-79.

5. Ben-Amotz, A. 1984. Development of outdoor raceway capable of yielding oil-rich halotolerant microalgae. Identification of oil-rich strains. In: *Aquatic Species Program Review: Proceedings of the April 1984 Principal Investigators Meetings.* Solar Energy Research Institute Publication SERI/CP-231-2341. pp. 186-195.

Nannochloropsis sp.

Strain: S/NANNO-2 (Nanno-Q)

Taxonomy: **Division:** Chrysophyta (1), Eustigmatophyta (2)
Class: Eustigmatophyceae
Order: Eustigmatales



Cells of *Nannochloropsis* sp. S/NANNO-2 (Scale: 1 cm = 2.5 μm)

Collection site: Marine water sample, Qingdao, China (R. Lewin)

Size: 2-3 μm

Growth form: unicells

Growth rate at optimum (or maximum recorded): 1.04 doublings day^{-1}

Culture conditions:

Vitamins required:	not determined
Available nitrogen sources:	ammonium, nitrate, urea
Suitable media:	GPM
Nutritional modes:	photoautotrophic
Temperature range:	11°-35°C
optimum:	24°C
Salinity range:	35-350 g TDS L^{-1} (3)
optimum:	200-300 g TDS L^{-1} (3)

Chemical composition:

Growth Conditions	% lipid	% protein	% carbohydrate	Ref.	Basis
Not specified (#80)	35.6			4	AFDW
Not specified (#81)	46.7			4	AFDW
Not specified (#82)	48.7			4	AFDW
Not specified (#83)	52.6			4	AFDW
B	31.4				AFDW
B,N(N, severe)	64.0				AFDW

Lipid composition:

Growth Conditions	Fraction eluted by:					Ref.
	Hexane	Benzene	Chloroform	Acetone	Methanol	
N.S. (#80)	3.9	27.7	32.6	21.3	14.4	4
N.S. (#81)	5.1	59.1	17.9	6.9	10.9	4
N.S. (#82)	4.9	65.8	17.4	7.5	4.4	4
N.S. (#83)	4.8	64.7	17.7	7.1	5.8	4
B,N(N)(early)	2.5	4.5	5.1	66.3	21.2	4
B,N(N)(late)	1.7	29.3	25.0	34.5	9.9	4

Physiological notes:

1. Grows over a pH range of 6-10, with an optimum near 9.0. (3)

2. New cultures usually exhibit a lag phase of 5-7 days when inoculated from a stationary phase culture.

Life cycle:

Knowlege of the life cycle of this organism is incomplete. Only asexual reproduction has been observed.

Literature cited:

1. Bold, H.C. & M.J. Wynne. 1978. *Introduction to the Algae.* Prentice-Hall, New Jersey. 706 pp.

2. Hibberd, D.L. 1981. Notes on the taxonomy and nomenclature of the algal classes Eustigmatophyceae and Tribophyceae (synony Xanthophyceae). *Bot. J. Limn. Soc.* 82: 93-119.

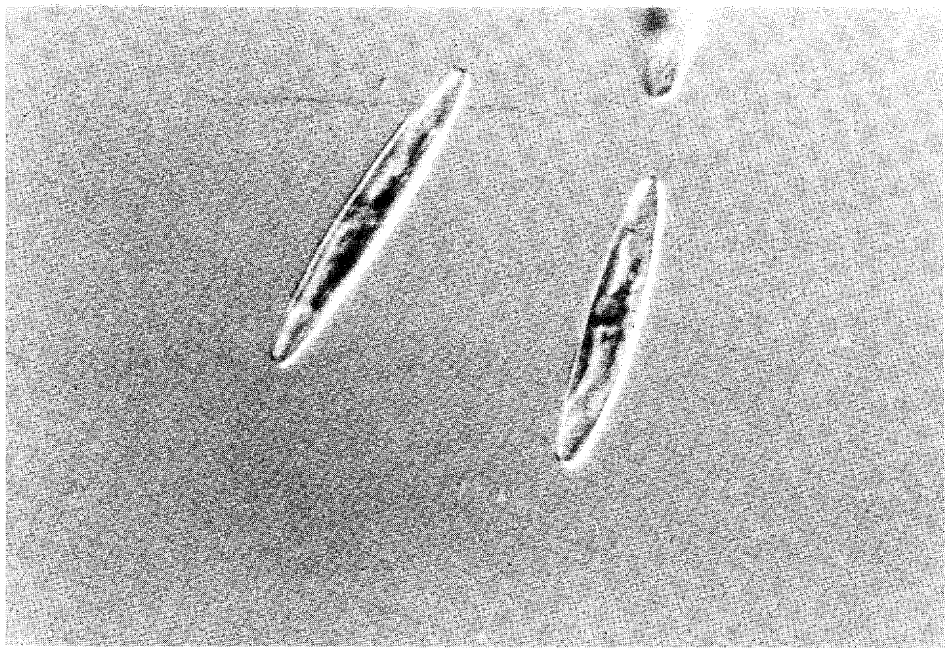
3. Lewin, R. Unpublished data.

4. Benemann, J. Unpublished data.

Nitzschia sp.

Strain: S/NITZS-1

Taxonomy: **Division:** Chrysophyta
Class: Bacillariophyceae
Order: Pennales
Family: Nitzschiaceae



Cells of *Nitzschia* sp. S/NITZS-1 (Scale: 1 cm = 10 μm)

Collection site: Mono Lake, California, USA (D. Chapman)

Size: 40-53 μm x 6-8 μm

Growth form: unicells

Growth rate at optimum (or maximum recorded): not determined

Culture conditions:

Vitamins required: none

Available nitrogen sources: nitrate (best), urea

Suitable media: Mono Lake

Nutritional modes: photoautotrophic

Temperature range: 10°-44°C (1)

optimum: 30°-36°C (1)

Salinity range: 30-90 g TDS L^{-1} (1)

optimum: 50-70 g TDS L^{-1} (1)

Chemical composition:

Growth Conditions	% lipid	% protein	% carbohydrate	Ref.	Basis
B	27	36	16	1	DW

Lipid composition:

Growth Conditions	Fraction eluted by:					Ref.
	Hexane	Benzene	Chloroform	Acetone	Methanol	
B	0.9	1.7	51.2	22	24.6	2

Fuel production options:

Option	Methane MJ/kg	Ester MJ/kg	Hydro-carbon MJ/kg	Ethanol MJ/kg	Total Recovered MJ/kg	Fraction Utilized	Liquid Fuel MJ/kg
1	13.6	0	0	0	13.6	0.702	0
2	13.6	0	0	0	13.6	0.702	0
3	6.8	5.9	0.1	0	12.8	0.662	6.0
4	12.1	0	0	1.4	13.4	0.693	1.4
5	5.3	5.9	0.1	1.4	12.7	0.653	7.4

Total energy content: 19.4 MJ/kg dry weight

Physiological notes:

Major fatty acids are 14:0, 14:1, 16:0, 16:1, 16:2, 16:3, 20:6. (3)

Life cycle:

In sexual reproduction, two conjugating cells each form two gametes. Union of the gametes through a conjugation tube connecting these cells results in the formation of two autospores.

Outdoor culture history:

1. *Nitzschia* spp. have been noted to be occasional dominant algae in seawater-enrichment cultures in Woods Hole, Massachusetts (4), and France. (5)

2. *Nitzschia longissima* occurred in heated mass culture units in France. (6)

3. *Nitzschia closterium* has been cultivated as a food organism for penaeid protozoea. (7)

Literature cited:

1. Thomas, W.H., D.L.R. Seibert, M. Alden, P. Eldridge, A. Neori & S. Gaines. 1983. Selection of high-yielding microalgae from desert saline environments. In: *Aquatic Species Program Review: Proceedings of the March 1983 Principal Investigators Meetings.* Solar Energy Research Institute Publication SERI/CP-231-1946. pp. 97-123.

2. Ben-Amotz, A. & T.G. Tornabene. 1983. Chemical profile of algae with emphasis on lipids of microalgae. In: *Aquatic Species Program Review: Proceedings of the March 1983 Principal Investigators Meeting.* Solar Energy Research Institute Publication SERI/CP-231-1946. pp. 123-135.

3. Tornabene, T.G. 1984. Chemical profile of microalgae with emphasis on lipids. *Aquatic Species Program Review: Proceedings of the April 1984 Principal Investigators Meetings.* Solar Energy Research Institute Publication SERI/CP-231-2341. pp. 64-78.

4. Goldman, J.C. & J.H. Ryther. 1976. Temperature influenced species -- competition in mass cultures of marine phytoplankton. *Biotech. & Bioeng.* 18:1125-1144.

5. Persoone, G., J. Morales, H. Verlet & N. De Pauw. 1980. Air-lift pumps and the effect of mixing on algal growth. In: *Algae Biomass.* Shelef, G. & C.J. Soeder (eds.). Elsevier/North-Holland Biomedical Press. pp. 505-522.

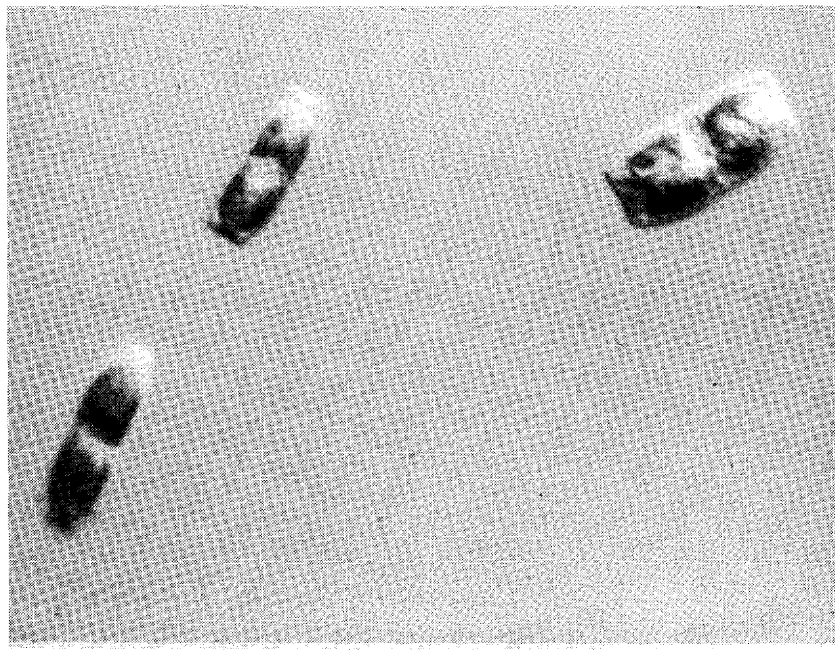
6. DePauw, N., H. Verlet & L. DeLeenheer. 1980. Heated and unheated outdoor cultures of marine algae with animal manure. In: *Algae Biomass.* Shelef, G. & C.J. Soeder (eds.). Elsevier/North-Holland Biomedical Press. pp. 315-341.

7. SEAFDEC. 1975. Studies on the Prawn. South East Asian Fishery Development Center. Aquaculture Dept. Annual Report. 1975. pp. 13-29.

Nitzschia dissipata

Strain: S/NITZS-2 (DI-160)

Taxonomy: **Division:** Chrysophyta
Class: Bacillariophyceae
Order: Pennales
Family: Nitzschiaceae



Cells of *Nitzschia dissipata* S/NITZS-2 (Scale: 1 cm = 8.5 μm)

Collection site: Dauphin Island, Gulf of Mexico (M. Tadros) (1)

Date: June 1984
Water temperature: 29°C
Salinity: 26 g TDS L^{-1}
pH: 8.0

Size: 15-35 μm

Growth form: unicells

Growth rate at optimum (or maximum recorded): 1.32 doublings day^{-1}

Culture conditions:

Vitamins required:	not determined
Available nitrogen sources:	nitrate
Suitable media:	f/2 (with 75 mg/L $NaNO_3$)
Nutritional modes:	photoautotrophic
Temperature range:	20°-30°C
optimum:	27°-28°C
Salinity range:	6-45 g TDS L^{-1} (2)
optimum:	32-45 g TDS L^{-1} (2)

Chemical composition:

Growth Conditions	% lipid	% protein	% carbohydrate	Ref.	Basis
B	26.3	20.2	29.4	1	AFDW
B,N(N)	66.0	12.6	9.3	1	AFDW

Physiological notes:

Growth inhibited by high concentrations of nitrate.

Life cycle:

Vegetative cell division is the ordinary method of reproduction. Sexual reproduction is isogamous. Amoeboid gametes fuse, resulting in the formation of an auxospore.

Literature cited:

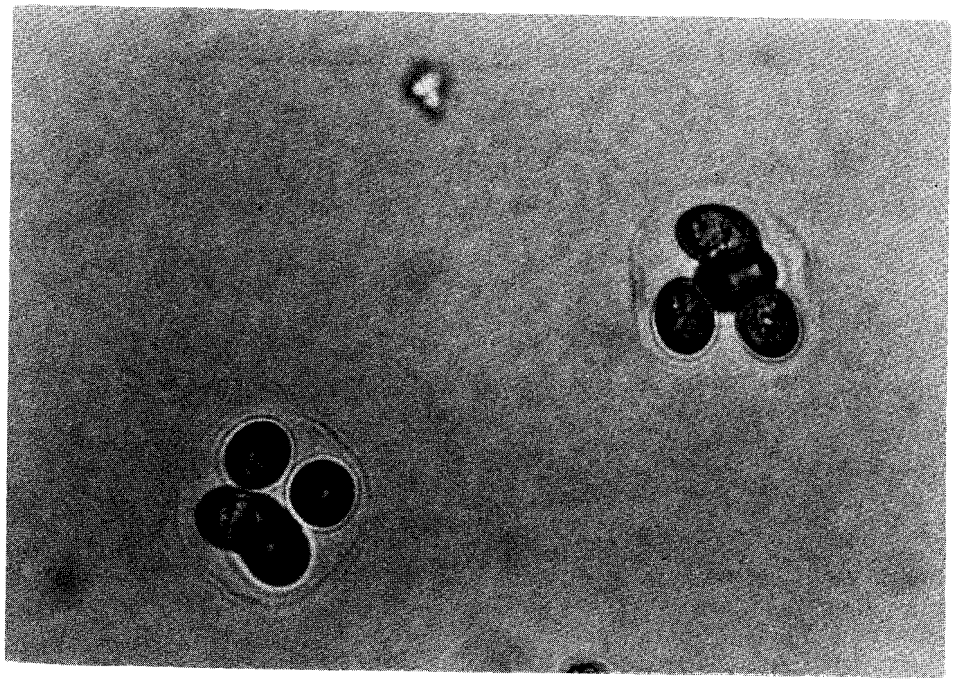
1. Tadros, M.G. 1985. Screening and characterizing oleaginous microalgal species from the southeastern United States. In: *Aquatic Species Program Review: Proceedings of the March 1985 Principal Investigators' Meeting.* Solar Energy Research Institute Publication SERI/CP-231-2700. pp. 83-99.

2. Tadros, M.G. Unpublished data.

Oocystis pusilla

Strain: S/OOCYS-1

Taxonomy: **Division:** Chlorophyta
Class: Chlorophyceae
Order: Chlorococcales
Family: Oocystaceae



Cells of *Oocystis pusilla* S/OOCYS-1 (Scale: 1 cm = 10 µm)

Collection site: Walker Lake, California, USA (W. Thomas) (1)

Date: October 1982
Water temperature: 18°C
Salinity: 10.6 o/oo
pH: 9.3

Size: individual cells = 11-14 µm x 8-10 µm

Growth form: unicells--two or three generations of cells may be enclosed within an original mother-cell wall which enlarges so that it often appears as a gelatinous sheath.

Growth rate at optimum: not determined

Culture conditions:

Vitamins required: not determined

Available nitrogen sources: urea, nitrate, ammonium

Suitable media: Walker Lake

Temperature range: 15°-33°C (1)

optimum: 25°-26°C (1)

Salinity range: 10-25 g TDS L^{-1} (1)

optimum: 18 g TDS L^{-1} (1)

Chemical composition:

Growth Conditions	% lipid	% protein	% carbohydrate	Ref.	Basis
B	10.5	39	37	2	AFDW

Fuel production options:

Option	Methane MJ/kg	Ester MJ/kg	Hydro-carbon MJ/kg	Ethanol MJ/kg	Total Recovered MJ/kg	Fraction Utilized	Liquid Fuel MJ/kg
1	13.0	0	0	0	13.0	0.675	0
2	13.0	0	0	0	13.0	0.675	0
3	9.8	0	0	0	9.8	0.506	0
4	8.9	0	0	3.6	12.6	0.652	3.6
5	5.7	0	0	3.6	9.3	0.483	3.6

Total energy content: 19.3 MJ/kg dry weight

Life cycle:

Reproduction is exclusively by the formation of autospores. The autospores can remain for some time in a greatly expanded parent cell wall.

Outdoor culture history:

Oocystis is an occasional dominant algae in algal mass culture systems integrated with wastewater treatment systems in Israel. (3)

Literature cited:

1. Thomas, W.H., D.L.R. Seibert, M. Alden & P. Eldridge. 1984. Cultural requirements, yields, and light utilization efficiencies of some desert saline microalgae. In: *Aquatic Species Program Review: Proceedings of the April 1984 Principal Investigators Meetings.* Solar Energy Research Institute Publication SERI/CP-231-2341. pp. 7-51.

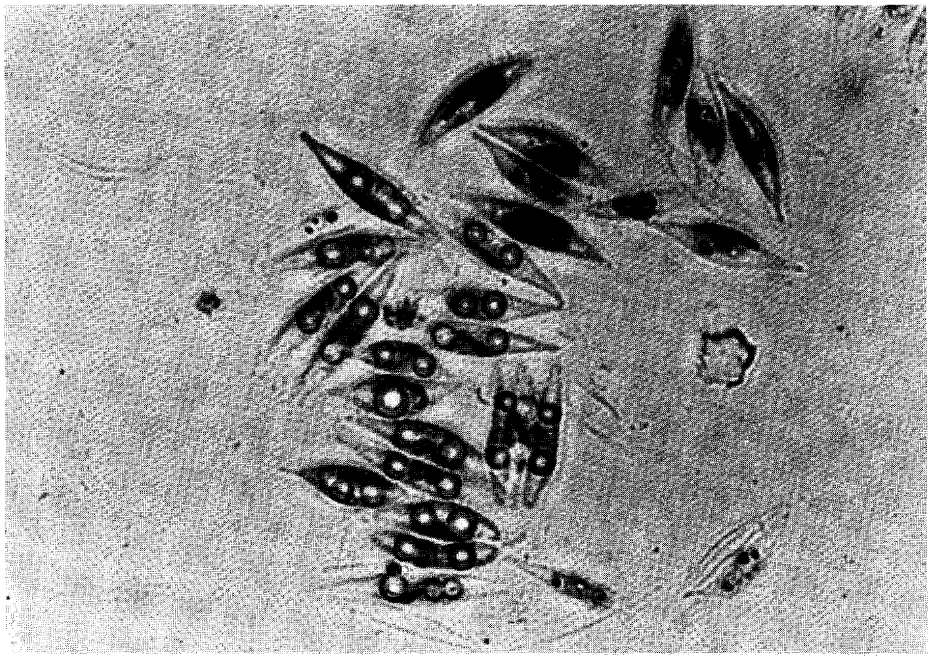
2. *SERI Aquatic Species Program 1983 Annual Report.* Solar Energy Research Institute Publication SERI/PR-231-2271.

3. Shelef, G., Y. Azov, R. Moraine & G. Oron. 1980. Algal mass production as an integral part of a wastewater treatment and reclamation system. In: *Algae Biomass.* Shelef, G. and C.J. Soeder, (eds.). Elsevier/North-Holland Biomedical Press. pp. 163-189.

Phaeodactylum tricornutum Bohlin

Strain: S/PHAEO-1 (TFX-1)

Taxonomy: **Division:** Chrysophyta
Class: Bacillariophyceae
Order: Pennales
Family: Phaeodactylaceae



Cells of ***Phaeodactylum tricornutum*** S/PHAEO-1 containing droplets of storage lipids. (Scale: 1 cm = 10 μm)

Collection site: Woods Hole, Massachusetts, USA

Size: 15-22 μm x 3-4 μm

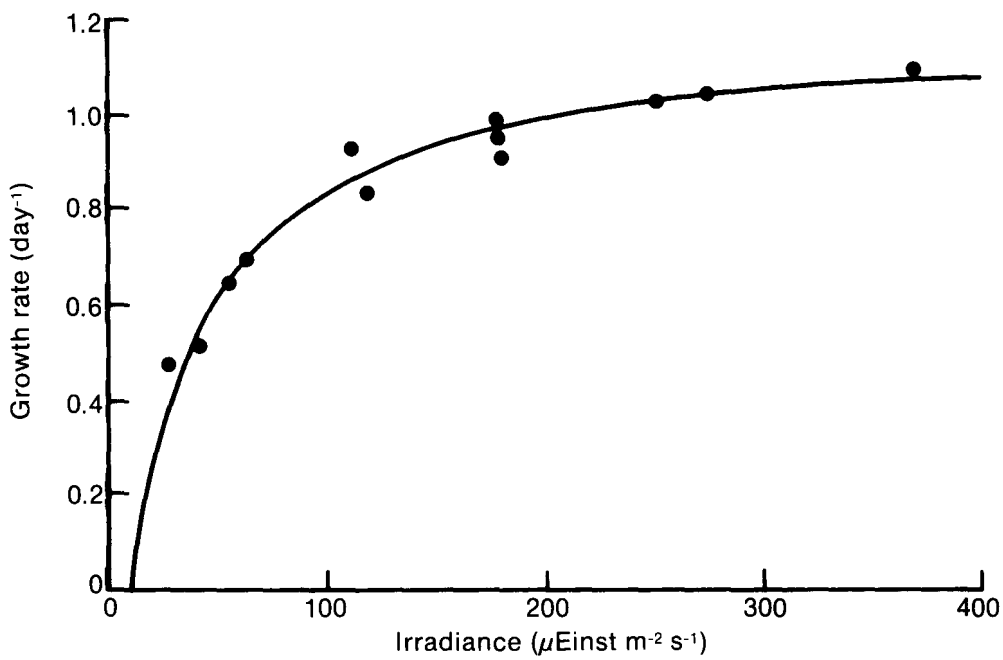
Growth form: unicells

Growth rate at optimum (or maximum recorded): 1.96 doublings day^{-1} (1)

Culture conditions:

Vitamins required:	none
Available nitrogen sources:	ammonium, nitrate, urea, many organics
Suitable media:	ASW, GPM, f/2
Nutritional modes:	photoautotrophic
Temperature range:	<15°-27°C (1)
optimum:	24°C (1)
Salinity range:	<20-70 g TDS L^{-1} (1)
optimum:	35 g TDS L^{-1} (1)

Light curve of growth:



at 25°C with light of 5600K color temperature, nitrogen suplied as NH_4^+. (1,2)

Chemical composition:

Extensive data are available on the biochemical composition of this species under various conditions. (1,2,3) The following data are typical:

Growth Conditions	% lipid	% protein	% carbohydrate	Ref.	Basis
SC	38.2	34.5	15.3	1,2	C
SC, L(28)	36.4	42.1	11.2	1,2	C
C(.271), N(P), 21.5°C	42.7	27.1	20.4	1	C
C(.235), N(P), 24.5°C	38.0	29.0	15.0	1,3	C
C(.266), N(N), 21.5°C	56.8	20.9	11.4	1	C
C(.132), N(N), 24.5°C	50.0	31.0	11.0	1,3	C

Fuel production options:

Option	Methane MJ/kg	Ester MJ/kg	Hydro-carbon MJ/kg	Ethanol MJ/kg	Total Recovered MJ/kg	Fraction Utilized	Liquid Fuel MJ/kg
1	21.9	0	0	0	21.9	0.757	0
2	21.9	0	0	0	21.9	0.757	0
3	4.3	17.2	5.7	0	27.2	0.941	22.9
4	20.6	0	0	1.1	21.7	0.752	1.1
5	3.0	17.2	5.7	1.1	27.1	0.937	24.0

Total energy content: 28.913 MJ/kg dry weight

Physiological notes:

1. 8%-12% ash content.
2. Physiological differences between strains BB and TFX-1 have been documented. (2)

Life cycle:

Asexually reproducing cells of this genus are pleomorphic exhibiting fusiform, triradiate, or oval shapes. The fusiform shape is the normal form, with the triradiate or oval cells arising under special environmental conditions. Low calcium culture media has been shown to induce the ovoid form. (4)

Outdoor culture history:

A. Other strains of *P. tricornutum* (see also Thomas strain BB)

1. *P. tricornutum* was cultured in meter-deep tanks in the late 1950s and early 1960s at Poole, England. (5,6,7,8) Production was 2-5 g C m^{-2} d^{-1}.

2. In the late 1970s in Belgium, outdoor cultures that were enriched with animal manure were dominated by *P. tricornutum* and *Skeletonema costatum* when culture temperatures were below 20°C. Production was 1-10 g DW m^{-2} d^{-1}. (9)

3. *P. tricornutum* has dominated 50,000 L outdoor algal cultures which are used for rearing and stocks of oysters and clams. (10)

4. When introduced into phytoplankton cultures based on deep ocean water in Brazil, *P. tricornutum* displaced populations of pennate diatoms that had previously occured. (11)

B. Strain TFX-1

This strain was isolated from culture ponds at Woods Hole, Massachusetts, USA. These ponds were operated with wastewater-seawater mixtures. The cultures were unseeded and were dominated by different species in different seasons; *P. tricornutum* was the dominant species at moderate temperatures (10°-23°C). These systems produced 1-6 g C m^{-2} d^{-1}. (12,13)

Literature cited:

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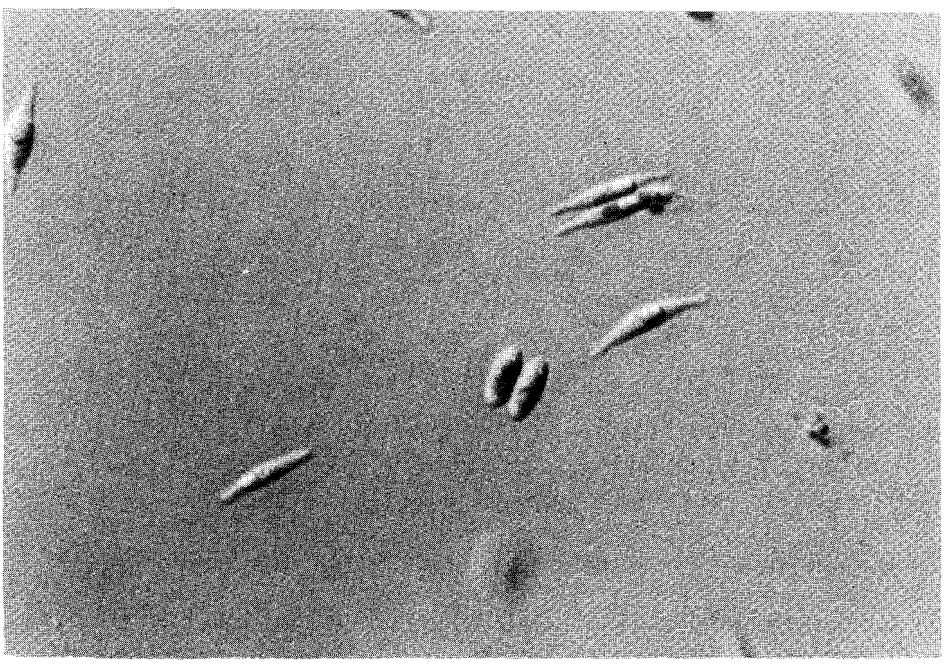
Phaeodactylum tricornutum **Bohlin**

Strain: S/PHAEO-2 (BB)

Taxonomy: **Division:** Chrysophyta
Class: Bacillariophyceae
Order: Pennales
Family: Phaeodactylaceae



Fusiform and ovoid cells of ***Phaeodactylum tricornutum*** S/PHAEO-2
(Scale: 1 cm = 10 μm)

Source: W. Thomas, Scripps Institution

Size: fusiform cells = 15 μm x 4 μm

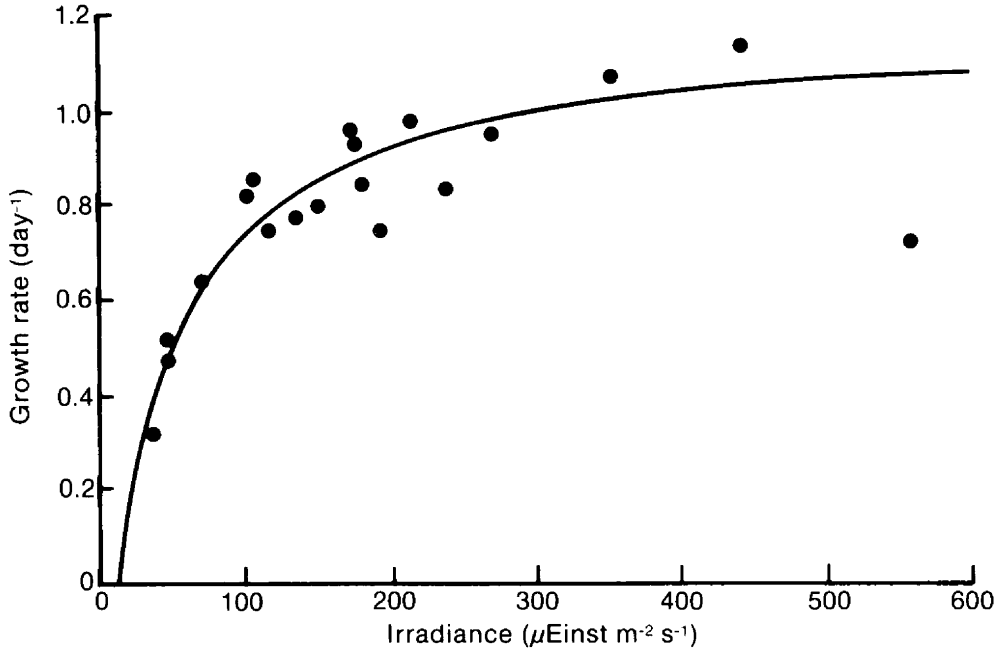
Growth form: unicells, chains (laterally attached)

Growth rate at optimum (or maximum recorded): 1.64 doublings day^{-1} (1)

Culture conditions:

Vitamins required:	none [(may be inhibitory (2)]
Available nitrogen sources:	ammonium, nitrate, urea, many organics
Suitable media:	ASW, GPM, f/2
Nutritional modes:	photoautotrophic
Salinity range:	<8.5-70 g TDS L^{-1} (3)
optimum:	35 g TDS L^{-1} (3)

Light curve of growth:



At 25°C with light of 5600K color temperature, nitrogen supplied as NH_4^+. (3,8)

Photoinhibition:

10% or more above ~ 500 μEinst m^{-2} s^{-1}.

Chemical composition:

Extensive data are available on the biochemical composition of this species under various conditions. (4,5,6,7) The following data are typical:

Growth Conditions	% lipid	% protein	% carbohydrate	Ref.	Basis
MC	24.6	----	----	4	C
C(.25), L	19.7	58.3	----	1	DW
C(.25), N(N)	23.2	19.7	----	1	DW
SC, L(.48)	34.2	45.3	9.5	3,8	C
SC	40.9	31.5	14.3	3,8	C

Fuel production options:

Option	Methane MJ/kg	Ester MJ/kg	Hydro-carbon MJ/kg	Ethanol MJ/kg	Total Recovered MJ/kg	Fraction Utilized	Liquid Fuel MJ/kg
1	18.3	0	0	0	18.3	0.722	0
2	18.3	0	0	0	18.3	0.722	0
3	6.9	11.1	3.7	0	21.7	0.858	14.8
4	16.8	0	0	1.3	18.1	0.715	1.3
5	5.4	11.1	3.7	1.3	21.5	0.852	16.1

Total energy content: 25.3 MJ/kg dry weight

Physiological notes:

Strain S/PHAEO-2 differs significantly from S/PHAEO-1 with respect to a large number of physiological parameters. (8)

Life cycle:

Asexually reproducing cells of this genus are pleomorphic exhibiting fusiform, triradiate or oval shapes. The fusiform shape is the normal form, with the triradiate or oval cells arising under special environmental conditions. Low calcium culture medium has been shown to induce the ovoid form. (9)

Outdoor culture history:

(See *P. tricornutum* S/PHAEO-1 for culture histories of other strains.)

1. A small (~0.5 m^2) shallow (2.2 cm) raceway system operated at Kaneoke, Hawaii, USA, in the mid 1970s gave a calculated production rate of 23 g AFDW m^{-2} d^{-1}. (10)

2. *P. tricornutum* S/PHAEO-2 was grown in a shallow raceway system in Hawaii. Achieved production of 25 g m^{-2} d^{-1} (photosynthetic efficiency 5%-6%), but temperature control was required to achieve species survival. (4,5,6,7)

Literature cited:

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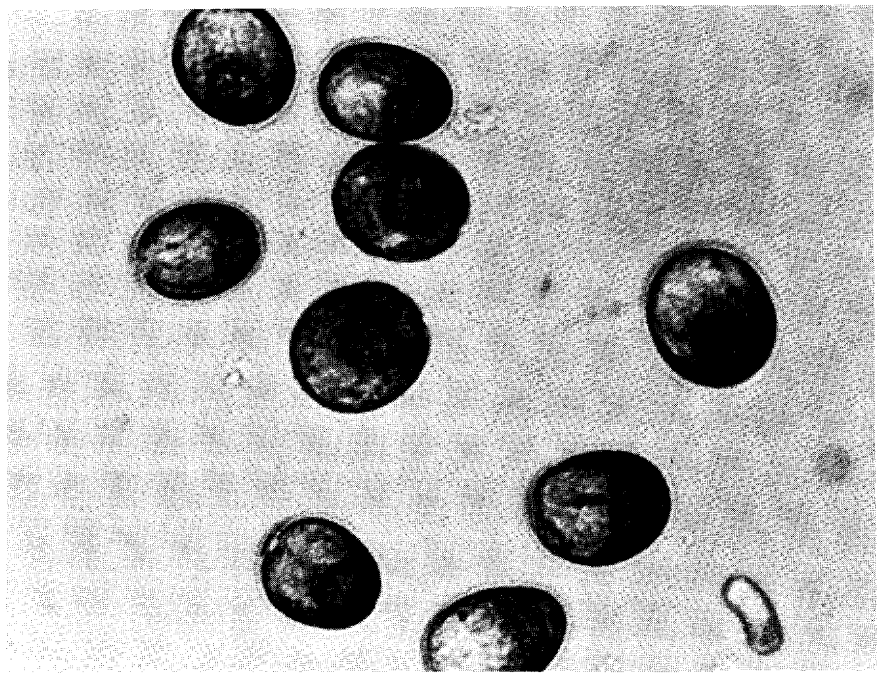
Tetraselmis sp.

Strain: S/PLATY-1

Taxonomy: **Division:** Chlorophyta
Class: Chlorophyceae
Order: Volvocales
Family: Tetraselmiaceae



Cells of *Tetraselmis* sp. S/PLATY-1 (Scale: 1 cm = 10 μm)

Collection site: Invaded raceway mass culture, Hawaii, USA (E. Laws)

Date: Summer 1983

Size: 13-18 μm x 13 μm

Growth form: unicellular

Growth rate at optimum (or maximum recorded): 2.1 doublings day^{-1} (1)

Culture conditions:

Vitamins required: none

Available nitrogen sources: ammonium, urea, nitrate, amino acids

Suitable media: Type I/10

Nutritional modes: autotrophic

Temperature range: not determined

optimum: 34°C (2)

Salinity range: 15->35 g TDS L^{-1} (1)

optimum: 35 g TDS L^{-1} (1)

Chemical composition:

Growth Conditions	% lipid	% protein	% carbohydrate	Ref.	Basis
SC	18	46	36	2	AFDW
SC, N(N,P)	15	24	61	2	AFDW

Lipid composition:

33% neutral lipids. (2)

Fuel production options:

Option	Methane MJ/kg	Ester MJ/kg	Hydro-carbon MJ/kg	Ethanol MJ/kg	Total Recovered MJ/kg	Fraction Utilized	Liquid Fuel MJ/kg
1	16.2	0	0	0	16.2	0.685	0
2	16.2	0	0	0	16.2	0.685	0
3	10.7	4.7	2.7	0	18.0	0.761	7.4
4	12.3	0	0	3.5	15.8	0.667	3.5
5	6.7	4.7	2.7	3.5	17.6	0.743	10.9

Total energy content: 23.7 MJ/kg dry weight

Physiological note:

Optimum pH = 7.0. (2)

Life cycle:

Asexual reproduction by longitudinal division to form two or four daughter cells. Some species of *Tetraselmis* are known to form resting spores or cysts. (3)

Outdoor culture history:

Cultured in Hawaii in a 48 m^2 raceway system. High productivity (35-45 g $m^{-2}d^{-1}$) at a salinity of 15-30 o/oo and at 28°-32°C. (1,2)

Literature cited:

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Appendix

CULTURE MEDIA

ASW Medium
(Darley and Volcani, 1969)

To 1 L of distilled water add:

NaCl	23.6 g
$MgSO_4 \cdot 7H_2O$	4.9 g
$MgCl_2 \cdot 6H_2O$	4.1 g
$CaCl_2$	1.1 g
KCl	75 mg
KNO_3	303 mg
Na_2EDTA	12 mg
$Na_2SiO_3 \cdot 9H_2O$	40 mg
glycylglycine	660 mg
thiamine - HCl	0.5 mg
trace elements	1.0 mL

Adjust to pH 8.0 before autoclaving. Autoclave separately 0.456 g K_2HPO_4 in 100 mL distilled water, and add 10 mL/L at time of inoculation.

Trace element stock (for 1 L):

H_3BO_3	0.568 g
$ZnCl_2$	0.624 g
$CuCl_2 \cdot 2H_2O$	0.268 g
$Na_2MoO_4 \cdot 2H_2O$	0.252 g
$CoCl_2 \cdot 6H_2O$	0.42 g
$FeSO_4$	1.36 g
$MnCl_2 \cdot 4H_2O$	0.36 g
Na-tartrate	1.77 g

Bolds Basal Medium
(Bischoff and Bold, 1963)

Six stock solutions (in distilled or deionized water) 400 mL in volume should be prepared, each containing one of the following salts in the concentration listed:

Salt	Grams
$NaNO_3$	10.0 g
$CaCl_2 \cdot 2H_2O$	1.0 g
$MgSO_4 \cdot 7H_2O$	3.0 g
K_2HPO_4	3.0 g
KH_2PO_4	7.0 g
NaCl	1.0 g

To 940 mL distilled water, add 10 mL of each stock solution and 1.0 mL of each of the stock trace-element solutions prepared as follows:

1. 50 g EDTA and 31 g KOH dissolved in 1 L distilled H_2O (or 50 g Na_2EDTA dissolved in 1 L distilled H_2O).

2. 4.98 g $FeSO_4 \cdot 7H_2O$ dissolved in 1 L of acidified water (acidified H_2O: 1.0 mL H_2SO_4 dissolved in 1 L distilled H_2O).

3. 11.42 g H_3BO_3 dissolved in 1 L distilled H_2O.

4. The following, in amounts indicated, all dissolved in 1 L distilled water: $ZnSO_4 \cdot 7H_2O$, 8.82 g; $MnCl_2 \cdot 4H_2O$, 1.44 g; MoO_3, 0.71 g; $CuSO_4 \cdot 5H_2O$, 1.57 g; $Co(NO_3)_2 \cdot 6H_2O$, 0.49 g.

Adjust to pH 7.0 before autoclaving.

Botryococcus Medium
(Ben-Amotz and Tornabene, 1983)

To 1 L of distilled water add:

$MgSO_4$	602 mg
$CaCl_2$	33 mg
KCl	373 mg
$NaHCO_3$	4201 mg
$Na_2SiO_3 \cdot 9H_20$	28 mg
H_3BO_3	6 mg
$FeCl_3$	0.4 mg
Na_2EDTA	11 mg
Tris	2420 mg
KNO_3	505 mg
KH_2PO_4	54 mg
Vitamin B_{12}	1.0 g
Thiamine-HCl	0.2 g
Biotin	1.0 g
f/2 trace elements stock	1.0 mL

Adjust to pH 8.0.

For f/2 trace elements stock solution, see f/2 seawater medium.

f/2 Seawater
(Guillard and Ryther, 1962)

To 1 L of filtered seawater add:

$NaNO_3$	75 mg
$NaH_2PO_4 \cdot H_2O$	5 mg
$Na_2SiO_3 \cdot 9H_2O$	30 mg
Thiamine-HCl	100 g
Biotin	0.5 g
B_{12}	0.5 g
Trace elements stock solution	1 mL

Trace elements stock solution (for 1 L):

Na_2EDTA	4.36 g
$FeCl_3 \cdot 6H_2O$	3.15 g
$MnCl_2 \cdot 4H_2O$	180 mg
$CuSO_4 \cdot 5H_2O$	10 mg
$ZnSO_4 \cdot 7H_2O$	22 mg
$CoCl_2 \cdot 6H_2O$	10 mg
$NaMoO_4 \cdot 2H_2O$	6 mg

Rila Marine Mix (Rila Products, Teaneck, New Jersey) can be substituted for the seawater. Dissolve 40 g of Rila Marine Mix in 1 L of distilled water. If Rila Marine Mix is used, 168 mg L^{-1} $NaHCO_3$ should also be added to the medium.

GPM Medium
(according to F. Haxo
Scripps Institution of Oceanography)

To 750 mL of filtered seawater (28-32 o/oo salinity) add the following:

distilled water	225 mL
KNO_3 (1M)	2 mL
K_2HPO_4 (1M)	0.2 mL
Soil extract	5 mL
PII trace metals	5 mL
B_{12} (1 µg/mL)	1 mL
Thiamine-HCl (1 mg/mL)	1 mL
Biotin (2 µg/mL)	1 mL

Autoclave the K_2HPO_4 addition separately in 10 mL of distilled water and add after the medium cools.

PII trace element stock (for 1 L):

Na_2EDTA	6.0 g
$FeCl_3 \cdot 6H_20$	0.29 g
H_3BO_3	6.84 g
$MnCl_2 \cdot 4H_2O$	0.86 g
$ZnCl_2$	0.06 g
$CoCl_2 \cdot 6H_2O$	0.026 g

Adjust trace element stock solution to pH 7.8-8.0 with NaOH.

Soil Extract:

> 1:1 wt. soil/volume distilled water. Autoclave and then fill with suction through Whatman No. 42 filter paper. Reautoclave filtered extract.

Rila Marine Mix (Rila Products, Teaneck, New Jersey) can be substituted for the seawater. Dissolve 30 g Rila Marine Mix in 750 mL of distilled water. If Rila Marine Mix is used, 168 mg L^{-1} $NaHCO_3$ should also be added to the medium. Additionally, 100-200 mg L^{-1} $NaSiO_3 \cdot 9H_2O$ should be added when culturing diatoms in this medium.

Modified Chu Medium
(Destordeur, Rossi & Sironval, 1982)

To 1 L of distilled water add:

KNO_3	200 mg
K_2HPO_4	20 mg
$MgSO_4 \cdot H_2O$	100 mg
$CaCl_2 \cdot 6H_2O$	80 mg
Fe citrate	20 mg
citric acid	100 mg
f/2 trace elements stock	1 mL

Adjust to pH 7.0 with KOH.

For f/2 trace elements stock solution, see f/2 seawater medium.

Mono Lake Medium
(according to W. Thomas
Scripps Institution of Oceanography)

To 1 L of distilled water add:

NaCl	26.30 g
Na_2CO_3	25.44 g
$NaHCO_3$	15.12 g
Na_2SO_4	14.20 g
KCl	2.91 g
H_3BO_3	1.92 g
KNO_3	1.01 g
$MgSO_4$	35 mg
Na_2SiO_3	198 mg
$Ca(NO_3)_2$	70 mg
KH_2PO_4	136 mg
Mono Lake trace elements stock	1 mL
1% Ferric Sequestrene	1 mL

Final pH should be adjusted to 9.3-9.7.

Trace elements stock (for 1 L):

$ZnSO_4 \cdot 7H_2O$	84 mg
H_3BO_3	600 mg
$CoCl_2 \cdot 6H_2O$	150 mg
$CuSO_4$	37 mg
$MnCl_2 \cdot 4H_2O$	400 mg
$(NH_4)_6Mo_7O_{24} \cdot 4H_2O$	370 mg

Pyramid Lake Medium
(according to W. Thomas
Scripps Institution of Oceanography)

To 1 L of distilled water add:

$NaCl$	3.271 g
$NaHCO_3$	1.176 g
$MgCl_2 \cdot 6H_2O$	508 mg
Na_2CO_3	392 mg
$CaCl_2$	28 mg
KCl	246 mg
Na_2SO_4	207 mg
$Na_2B_4O_7 \cdot 10H_2O$	9 mg
NaF	11 mg
$NaNO_3$	849 mg
KH_2PO_4	136 mg
1% Fe Sequestrene	1 mL
Mono Lake trace elements	1 mL

Final pH should be adjusted to 9.3-9.7.

For Mono Lake trace elements solution, see Mono Lake medium.

SERI Type I
Artificial Inland Saline Water

Recipes are provided for the preparation of Type I media at five different salinities, expressed as conductivity of the final solution. Formulas for these media were developed by statistical analysis of saline groundwater data for the state of New Mexico (Barclay et al., in preparation). For each salt, necessary additions in mg L^{-1} are listed.

	Conductivity (mmho cm^{-1})				
Salt	10	25	40	55	70
$CaCl_2$	0	3,932	5,618	7,610	8,430
$MgCl_2 \cdot 6H_2O$	4,114	11,844	22,789	35,305	42,230
Na_2SO_4	0	2,925	3,310	3,705	3,620
KCl	194	407	662	960	1,186
$NaHCO_3$	184	168	168	168	168
NaCl	2,118	3,845	9,132	13,023	16,039
$CaSO_4$	1,686	0	0	0	0

Suggested enrichments (mL/L) are:

Nitrogen source* (1 g-atom N L^{-1})	0.5 mL
K_2HPO_4 (1M)	0.5 mL
PII trace metals (see GPM medium)	5 mL
B_{12} (1 mg L^{-1})	1 mL
Thiamine-HCl (1 mg L^{-1})	1 mL
Biotin (2 mg L^{-1})	1 mL

*Nitrogen source indicated for individual species, ammonium as NH_4Cl, nitrate as KNO_3.

100-200 mg L^{-1} $Na_2SiO_3 \cdot 9H_2O$ should be added when cultivating diatoms in this medium.

SERI Type II
Artificial Inland Saline Water

Recipes are provided for the preparation of Type II media at five different salinities, expressed as conductivity of the final solution. Formulas for these media were developed by statistical analysis of saline groundwater data for the state of New Mexico (Barclay et al., in preparation). For each salt, necessary additions in mg L^{-1} are listed.

	Conductivity (mmho cm^{-1})				
Salt	10	25	40	55	70
$CaCl_2$	28	28	28	28	28
$MgCl_2 \cdot 6H_2O$	1,953	3,026	3,920	4,362	4,230
Na_2SO_4	2,671	5,870	15,720	23,305	28,360
KCl	466	965	2,028	3,044	3,673
$NaHCO_3$	1,208	2,315	2,855	3,234	3,245
Na_2CO_3	231	876	1,234	1,492	1,527
$CaSO_4$	1,511	8,078	12,963	20,588	26,075

Suggested enrichments (mL/L) are:

Nitrogen source* (1 g-atom N L^{-1})	0.5 mL
K_2HPO_4 (1M)	0.5 mL
PII Trace Metals (see GPM medium)	5 mL
B_{12} (1 mg L^{-1})	1 mL
Thiamine-HCl (1 mg L^{-1})	1 mL
Biotin (2 mg L^{-1})	1 mL

*Nitrogen source indicated for individual species, ammonium as NH_4Cl, nitrate as KNO_3.

100-200 mg L^{-1} $Na_2SiO_3 \cdot 9H_2O$ should be added when cultivating diatoms in this medium.

Walker Lake Medium
(according to W. Thomas Scripps Institution of Oceanography)

To 1 L of distilled water add:

$NaCl$	4.075 g
$NaHCO_3$	2.184 g
Na_2CO_3	1.322 g
Na_2SO_4	3.392 g
$CaCl_2$	28 mg
$MgSO_4 \cdot 7H_2O$	790 mg
KCl	430 mg
$Na_2B_4O_7 \cdot 10H_2O$	169 mg
NaF	9 mg
$NaNO_3$	849 mg
KH_2PO_4	136 mg
1% Fe Sequestrene	1 mL
Mono Lake trace elements	1 mL

For Mono Lake trace elements solution, see Mono Lake medium.

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