Microalgae Culture Collection

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Research Group**

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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 and Municipal Waste Technology 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.

All of the newly added strains have been recently isolated by SERI and its subcontractors in organized 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 strains added to the collection this year have been isolated from inland saline waters and marine waters. 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. Most of the strains are currently nonaxenic. **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.

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 $\times$ 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.

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 $\mu\text{E m}^{-2} \text{s}^{-1}$
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 coffeiformis Agardh

Strain: AMPHO1

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

Cells of *Amphora coffeiformis* AMPHO1

(a) living cells; (b) cleared valve

Scale: (a) 1 cm = 10 μm ; (b) 1 cm = 5 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: unicellular

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 GPM
Nutritional modes:	autotrophic
Temperature range:	20° to >35°C
Salinity range:	<10 to >70 mmho cm ⁻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% O₂ saturation relative to air, growth is reduced only 5% below that of a population maintained at air 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 as PAR) (1).

Literature cited:

1. Weissman, J. Unpublished data.
2. Benemann, J. Unpublished data.

Amphora sp.

Strain: AMPHO27 (MLS-1, ASU 0032)

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

Cells of *Amphora* sp. AMPHO27
(a) living cells; (b) cleared valves
Scale: (a) 1 cm = 10 μ m; (b) 1 cm = 5 μ m

Collection site: -Mirror Lake South, Chaves County, New Mexico, USA (M. Sommerfeld)

Date: July 18, 1985
Water temperature: 27°C
Salinity: 14.55 mmho cm⁻¹ conductivity
pH: 8.0

Size: 12 μ m $\times$ 4 μ m

Growth form: unicellular

Growth rate at optimum (or maximum recorded): 1.06 doublings day⁻¹

Culture conditions:

Available nitrogen sources:	nitrate
Suitable media:	SERI Type II, 25 mmho cm ⁻¹
Temperature:	26°C
Salinity range:	5-70 mmho cm ⁻¹
optimum:	10 mmho cm ⁻¹

Chemical composition:

Not determined, but large lipid droplets visible with Nile Red.

Life cycle:

Vegetative division is the common reproductive method. The parental cell forms two new cells that are slightly smaller than the parental cell.

Amphora sp.

Strain: AMPHO28 (GR-2, ASU3001)

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

Cells of *Amphora* sp. AMPHO28
(a) living cells; (b) cleared valves
Scale: (a) 1 cm = 10 μm ; (b) 1 cm = 5 μm

Collection site: Gila River, Graham County, Arizona, USA (M. Sommerfeld)

Date: June 16, 1986
Water temperature: 35°C
Salinity: 7.62 mmho cm^{-1} conductivity
pH: 8.4

Size: 21 μm $\times$ 9 μm

Growth form: unicellular

Growth rate at optimum (or maximum recorded): 0.848 doublings day^{-1}

Culture conditions:

Available nitrogen sources: nitrate

Suitable media: SERI Type II, 40 mmho cm⁻¹

Temperature: 26°C

Salinity range: 10-70 mmho cm⁻¹

optimum: 70 mmho cm⁻¹

Chemical composition:

Not determined, but large lipid droplets visible with Nile Red.

Life cycle:

Vegetative division is the common reproductive method. The parental cell forms two new cells that are slightly smaller than the parental cell.

Ankistrodesmus falcatus (Corda) Ralfs

Strain: ANKIS1 (Pyramid Lake, 91-1)

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

Cells of *Ankistrodesmus falcatus* ANKIS1
Scale: 1 cm = 10 μ m

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 μ m $\times$ 3 μ m

Growth form: unicellular

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) (2,5).*
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 Meeting*. 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 Meeting*. 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 Meeting*. 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 hooglandii Nicolai and Baas Becking

Strain: BOEKE1

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

Cells of *Boekelovia* sp. BOEKE1
(a-d) vegetative cells; (e) statocysts
Scale: (a,b,e) 1 cm = 5 μ m; (c,d) 1 cm = 10 μ m

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 μ m

Growth form: unicellular

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 mmho cm ⁻¹
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)

C)

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:					Ref.
	Hexane	Benzene	Chloroform	Acetone	Methanol	
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:

- Shows improved growth in high-bicarbonate media.
- Has a high nitrogen requirement [Q_{0N} is about $0.08 \text{ mol N (mol C)}^{-1}$].
- 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. Statocysts have been observed in both laboratory cultures and sediments from the original collecting site.

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 Kuetzing

Strain: BOTRY1 (UTEX #572)

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

Colony of *Botryococcus braunii* BOTRY1
Scale: 1 cm = 10 μ m

Source: Univ. of Texas culture collection

Size: individual cells = 11-12 μ m $\times$ 8-10 μ m

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 Meeting*. 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 *Botryococcus 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: CHAET1 (S/CHAET-1)

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

Cells of *Chaetoceros gracilis* CHAET1
Scale: 1 cm = 10 μ m

Source: R. York, Hawaii Institute of Marine Biology, Kaneohe, Hawaii, USA
(received from P. Bienfang, Oceanic Institute, Hawaii)

Size: 5-7 μ m $\times$ 4 μ m (setae 30-37 μ m)

Growth form: unicellular, 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 μ M (1).
4. Tolerates high oxygen concentrations. The growth rate at 500% O₂ saturation relative to air is reduced by 15%-25% from that observed at O₂ equilibrium (1).

Taxonomic note:

Recent observation of resting spores indicates that this strain may belong to *C. muelleri*.

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).
5. *C. gracilis* (CHAET1) was cultivated in 1.4 m² ponds in California. Production averaged 30 g m⁻² d⁻¹ (1).

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 protozoan larvae. *Aquaculture* 14: 105-113.
4. Hirata, J. Unpublished data.
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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 muelleri var. *subsalsum* (Lemm.) Johan. and Rush.

Strain: CHAET6

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

Cells of *C. muelleri* var. *subsalsum* CHAET6
(a) vegetative cell; (b) resting spore in mother cell
Scale: 1 cm = 5 μ m

Collection site: near Alamogordo, New Mexico, USA (W. Barclay)

Date: May 7, 1985
Water temperature: 27°C
Salinity: 2.6 mmho cm⁻¹
pH: 8.6

Size: valves 3–7 μ m $\times$ 3.5–13 μ m, perivalvar length 3.5–10.5 μ m

Growth form: unicellular

Growth rate at optimum: 3.39 doublings day⁻¹

Culture conditions:

Vitamins required:	not determined
Available nitrogen sources:	urea
Suitable media:	SERI Type II
Nutritional modes:	autotrophic
Temperature range:	10°-35°C
optimum:	25°-30°C
Salinity range:	10-70 mmho cm ⁻¹
optimum:	25-40 mmho cm ⁻¹

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.

Outdoor culture history:

Chaetoceros muelleri var. *subsalsum* (CHAET6) was cultivated in 1.4 m² outdoor ponds in Vacaville, California. Productivity averaged 35 g m⁻² d⁻¹ (1).

Literature cited:

1. Weissman, J. Unpublished data.

Chaetoceros muelleri var. *subsalsum* (Lemm.) Johan. and Rush.

Strain: CHAET10 (S/CHAET-4 UT-27)

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

Cells of *C. muelleri* var. *subsalsum* CHAET10
(a) vegetative cell; (b) resting spore
Scale: 1 cm = 10 μ m

Collection site: Antelope Island, Great Salt Lake, Utah, USA (S. Rushforth)

Date: June 18, 1985
Water temperature: 30°C
Salinity: 74000 mmho cm⁻¹

Size: apical length 4-12 μ m, perivalvar length 4.5-11.5 μ m, setae length 5-22 μ m

Growth form: unicellular

Growth rate at optimum (or maximum recorded): 4.0 doublings day⁻¹

Culture conditions:

Vitamins required:	not determined
Available nitrogen sources:	urea
Suitable media:	SERI Type II, 25 mmho cm^{-1}
Nutritional modes:	autotrophic
Temperature range:	13° to >35°C
optimum:	>35°C
Salinity range:	<10 to >70 mmho
optimum:	growth rate was independent of salinity over the range 10-70 mmho cm^{-1}

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.

Chemical composition:

Growth Conditions	% lipid	% protein	% carbohydrate	Ref.	Basis
B	18				AFDW
B,N(N)	38				AFDW
BN(Si)	39				AFDW

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:

Chaetoceros muelleri var. *subsalsum* was grown in 1.4 m² ponds in California. Production averaged 35 g m⁻² d⁻¹ (1).

Literature cited:

1. Weissman, J. Unpublished data.

Chaetoceros muelleri Lemmermann

Strain: CHAET14 (S/CHAET-2, SS-14)

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

Cell of *Chaetoceros muelleri* CHAET14
Scale: 1 cm = 2.5 μ m

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

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

Size: 6 μ m $\times$ 4 μ m

Growth form: unicellular, 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 m² ponds at Vacaville, California, during August and September 1985 in artificial seawater with pH controlled at 7.5-8.0 by automated CO₂ 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.

Chaetoceros muelleri Lemmermann

Strain: CHAET15 (49-1A)

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

Cells of *C. muelleri* CHAET15
(a) apical view of living cell;
(b) girdle view of cleared resting spore
Scale: (a) 1 cm = 10 μ m; (b) 1 cm = 5 μ m

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

Date: October 22, 1982
Water temperature: 25°C
Salinity: 2.8 TDS g L⁻¹

Size: apical length 4-9 μ m, perivalvar length 5-12 μ m, setae length 9-25 μ m

Growth form: unicellular

Growth rate at optimum: 1.73 doublings day⁻¹

Culture conditions:

Vitamins required: not determined

Available nitrogen sources: urea

Suitable media: GPM

Nutritional modes: autotrophic

Temperature range: 15°-30°C

optimum: 25°-30°C

Salinity range: 10-40 mmho cm⁻¹

optimum: 10 mmho cm⁻¹

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.

Strain: CHAET38 (SH9-1)

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

Cells of *C. muelleri* var. *subsalsum* CHAET38
Scale: 1 cm = 10 μ m

Collection site: Aquatic Farms, Pond 9, Oahu, Hawaii, USA 20° 30' 33.7" N, 157° 51' 23.5" W (R. York)

Date: July 10, 1985
Water temperature: 31.5°C
Salinity: 32.7 mmho cm⁻¹
pH: 7.4
Dissolved oxygen: 12.8 ppm

Size: 4-7.5 μm $\times$ 6-12 μm (setae 28-38 μm)

Growth form: unicellular

Growth rate (maximum recorded): 2.12 doublings day^{-1}

Production (maximum recorded): 31 g DW $\text{m}^{-2} \text{d}^{-1}$

Culture conditions:

Vitamins required: not determined

Available nitrogen sources: ammonium, nitrate

Suitable media: f/2, GPM

Nutritional modes: autotrophic

Temperature range: 17.7°-42.8°C

Salinity range: 32.7-45.0 mmho cm^{-1}

Chlorella sp.

Strain: CHLOR1 (SO1)

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

Cells of *Chlorella* sp. CHLOR1
Scale: 1 cm = 10 μ m

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 μ m exponential growth, 10-20 μ m stressed (1)

Growth form: unicellular

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 $30 \text{ g m}^{-2}\text{d}^{-1}$ (5).
3. Production of *Chlorella* in Asia exceeds 1000 kg of dried microalgae per month with average yield of $25\text{-}30 \text{ g m}^{-2}\text{d}^{-1}$ (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 Meeting*. 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 Meeting*. 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 Gerneck

Strain: CHLOR2 (BL-6)

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

Cells of *Chlorella ellipsoidea* CHLOR2
Scale: 1 cm = 3.8 μ m

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 μ m $\times$ 4 μ m

Growth form: unicellular

Growth rate at optimum (or maximum recorded): 5.3 doublings day⁻¹ (outdoors)

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 to >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 $\text{m}^{-2} \text{d}^{-1}$ 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: CHLOR3 (SC-2)

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

Cells of *Chlorella* sp. CHLOR3
Scale: 1 cm = 3.8 μ m

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 μ m $\times$ 4 μ m ovals

Growth form: unicellular

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° to >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:

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.

Cyclotella cryptica Reimann et al.

Strain: CYCLO1 (DI-35)

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

Cells of *C. cryptica* CYCLO1
(a) living cells; (b) cleared valves
Scale: (a) 1 cm = 10 μ m; (b) 1 cm = 5 μ m

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 μ m

Growth form: unicellular

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° to >35°C (1)
optimum:	30°C (1)
Salinity range:	6 to >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 m² 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.

Cyclotella cryptica Reimann et al.

Strain: CYCLO2

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

Cells of *C. cryptica* CYCLO2
(a) living cells; (b) cleared valve
Scale: (a) 1 cm = 10 μ m; (b) 1 cm = 5 μ m

Collection site: Inland pond, Key West, Florida, USA (P. Roessler)

Date: November 1984

Size: 5-10 μ m in diameter

Growth form: unicellular

Growth rate at optimum (or maximum recorded): 1.6 doublings day⁻¹

Culture conditions:

Vitamins required:	not determined
Available nitrogen sources:	urea
Suitable media:	Type I, 40 mmho cm^{-1}
Nutritional modes:	autotrophic
Temperature range:	16° to >35°C
optimum:	32°C
Salinity range:	<10 to >70 mmho cm^{-1}
optimum:	growth rate independent of salinity from 10-70 mmho cm^{-1}

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)

C)

Chemical composition:

Growth Conditions	% lipid	% protein	% carbohydrate	Ref.	Basis
B	24				AFDW
B, N(N)	22				AFDW
B, N(Si)	25				AFDW

Taxonomic notes:

This strain is unusual for *C. cryptica* in that the central strutted process is usually absent.

Life cycle:

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

Ellipsoidon sp.

Strain: ELLIP1 (70-01)

Taxonomy: Division: Eustigmatophyta
 Class: Eustigmatophyceae
 Order: Eustigmatales (?)
 Family: Pleurochloridaceae

Cells of *Ellipsoidon* sp. ELLIP1
Scale: 1 cm = 10 μ m

Collection site: Curacao, Netherlands Antilles (R. Lewin)

Date: February 1985

Size: 4-8 μ m $\times$ 2 μ m

Growth form: unicellular

Culture conditions:

Vitamins required:	none
Available nitrogen sources:	nitrate
Suitable media:	GPM
Nutritional modes:	autotrophic

Chemical composition:

Growth Conditions	% lipid	% protein	% carbohydrate	Ref.	Basis
B, N(N)	50				AFDW

Physiological notes:

1. Chlorophyll a/b ratio = 6.71 (1).
2. Grows well at salinities from 0.5 to 2 times seawater (1).

Literature cited:

1. Lewin, R. Unpublished data.

Franceia sp.

Strain: FRANCI (LCC-1, ASU 0146)

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

(a) and (b) Cells of *Franceia* sp. FRANCI
Scale: 1 cm = 10 μ m

Collection site: Lower Chevelon Creek, Navajo County, Arizona, USA (M. Sommerfeld)

Date: July 27, 1985
Water temperature: 23°C
Salinity: 2.80 mmho cm⁻¹ conductivity
pH: 7.8

Size: 5-7 μ m $\times$ 4-5 μ m

Growth form: unicells

Growth rate at optimum (or maximum recorded): 1.58 doublings day⁻¹

Culture conditions:

Available nitrogen sources:	nitrate
Suitable media:	SERI Type II, 5 mmho cm ⁻¹
Temperature range:	26°C
Salinity range:	5-70 mmho cm ⁻¹
optimum:	10 mmho cm ⁻¹

Life cycle:

Reproduction by formation of two, four, or eight autospores that separate from one another.

Chemical composition:

Growth Conditions	% lipid	% protein	% carbohydrate	Ref.	Basis
B	26.5	60.6	61.0	-	AFDW

Isochrysis aff. *galbana* Green

Strain: ISOCH1 (Tahitian T-ISO)

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

Cells of *Isochrysis* aff. *galbana* ISOCH1
Scale: 1 cm = 5 μ m

Source: R. York, Hawaii Institute of Marine Biology, Kaneohe, Hawaii, USA
(Received from Jean-Louis Marten, Aquacop Co., Tahiti. Bloomed in aquaculture tank.)

Size: 7-4 μ m $\times$ 4 μ m

Growth form: unicellular flagellate

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:	photoautotrophic
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:					Ref.
	Hexane	Benzene	Chloroform	Acetone	Methanol	
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 to 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 Meeting*. 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 Meeting*. 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: MONORI

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

Cells of *Monoraphidium* sp. MONOR1
Scale: 1 cm = 2.5 μ m

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 $\times$ 2 μm

Growth form: unicellular

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:	SERI Type II, 25 mmho cm^{-1}
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.

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:

1. Benemann, J. Unpublished data.

Monoraphidium sp.

Strain: MONOR2

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

Cells of *Monoraphidium* sp. MONOR2
Scale: 1 cm = 2.5 μ m

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 μ m $\times$ 2 μ m

Growth form: unicellular

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: SERI Type I, 10 mmho cm^{-1}
 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:

MONOR2 was cultured outdoors at Vacaville, California, during September 1985 in a low salinity Type I medium, and showed a production rate of $15\text{--}20 \text{ g m}^{-2} \text{ d}^{-1}$ (1).

Literature cited:

1. Weissman, J. Unpublished data.

Nannochloris sp.

Strain: NANNO2

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

Cells of *Nannochloris* sp. NANNO2
Scale: 1 cm = 10 μ m

Collection site: Peace Corp Pond, Harbor Branch Foundation, Fort Pierce, Florida, USA (R. Carlson)

Date: June 1985
Salinity: 25 ppt

Size: 4 μ m

Growth form: unicellular

Growth rate at optimum (or maximum recorded): 3.21 doublings day⁻¹

Culture conditions:

Vitamins required:	not determined
Available nitrogen sources:	nitrate
Suitable media:	f/2
Nutritional modes:	photoautotrophic
Temperature range:	17°-35°C
optimum:	30°C
Salinity range:	8-59 mmho cm ⁻¹
optimum:	22 mmho cm ⁻¹

Temperature/salinity growth responses:

Exponential growth rate (doublings day⁻¹) in batch culture.

Water Type	Growth (doublings day ⁻¹)
Seawater, 25 mmho cm ⁻¹	1.8
Seawater, 40 mmho cm ⁻¹	2.0
SERI Type I, 25 mmho cm ⁻¹	2.1
SERI Type I, 40 mmho cm ⁻¹	2.0
SERI Type II, 25 mmho cm ⁻¹	2.2
SERI Type II, 40 mmho cm ⁻¹	2.1

Growth response in batch culture of *Nannochloris* sp. to temperature and salinity. Contours are exponential growth rate (doublings day⁻¹).

Chemical composition:

Growth Conditions	% lipid	% protein	% carbohydrate	Ref.	Basis
B	30.3	12.2	35.5		AFDW
B, N(N)	56.3	3.2	23.0		AFDW

Life cycle:

Vegetative reproduction by the formation of autospores.

Outdoor culture history:

Nannochloris sp. was grown in full-strength seawater in 1.4 m² ponds in California. Achieved production of 19 g m⁻² d⁻¹ (photosynthetic efficiency 4.6%) over a period of 20 days (1).

Literature cited:

1. Weissman, J. Unpublished data.

Nannochloris sp.

Strain: NANNO12

Taxonomy: Division: Chlorophyta
 Class: Chlorophyceae
 Order: Tetrasporales
 Family: Coccomyxaceae

Cells of *Nannochloris* sp. NANNO12
Scale: 1 cm = 10 μ m

Collection site: Miami, Florida, USA (R. Lewin)

Date: February 1985

Size: <2 μ m

Growth form: unicellular

Culture conditions:

Vitamins required:	none
Available nitrogen sources:	nitrate
Suitable media:	GPM
Nutritional modes:	autotrophic

Chemical composition:

Growth Conditions	% lipid	% protein	% carbohydrate	Ref.	Basis
B, N(N)	60			1	AFDW

Physiological notes:

1. Chlorophyll a/b ratio = 1.72 (1).
2. Grows well at temperatures at least up to 36°C (1).
3. Exhibits good growth in salinities from 0 to 2 times seawater (1).

Literature cited:

1. Lewin, R. Unpublished data.

Nannochloropsis salina Hibberd

Strain: NANNP1 (GBSTICHO)

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

Cells of *Nannochloropsis salina* NANNP1
Scale: 1 cm = 5 μ m

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

Date: 1952

Size: 2.5–5 μ m $\times$ 1.5–1.7 μ m

Growth form: unicellular

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 Meeting*. 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 Meeting*. 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 Meeting*. Solar Energy Research Institute Publication SERI/CP-231-2341. pp. 186-195.

Nannochloropsis sp.

Strain: NANNP2 (Nanno-Q)

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

Cells of *Nannochloropsis* sp. NANNP2
Scale: 1 cm = 2.5 μ m

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

Size: 2-3 μ m

Growth form: unicellular

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:

Knowledge 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.

Navicula saprophila Lange-Bertalot and Bonik

Strain: NAVIC1

Taxonomy: Division: Chrysophyta
 Class: Bacillariophyceae
 Order: Pennales
 Family: Naviculaceae

Cells of *N. saprophila* NAVIC1
(a) living cells; (b) cleared valve
Scale: (a) 1 cm = 10 μ m; (b) 1 cm = 5 μ m

Collection site: Lower Sugar Loaf Key, Key West, Florida, USA (P. Roessler)

Date: November 1984

Size: valves 5.5-7 μ m long $\times$ 3-4 μ m wide

Growth form: unicellular

Growth rate at optimum (or maximum recorded): 4.0 doublings day⁻¹

Culture conditions:

Vitamins required: not determined

Available nitrogen sources: urea

Suitable media: SERI Type II, 25 mmho cm⁻¹

Nutritional modes: autotrophic

Temperature range: 13° to >35°C

optimum: 30°C

Salinity range: <10 to >70 mmho cm⁻¹

optimum: 10 to 40 mmho cm⁻¹

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.

Chemical composition:

Growth Conditions	% lipid	% protein	% carbohydrate	Ref.	Basis
B	22				AFDW
B,N(N)	58				AFDW
B,N(Si)	49				AFDW

Physiological notes:

Cells are very sticky below pH 9.0.

Outdoor culture history:

Attempts to cultivate *Navicula saprophila* (NAVIC1) in outdoor culture systems in Vacaville, California, and in Hawaii have been unsuccessful. The cells are very sticky and do not remain in suspension (1,2).

Literature cited:

1. Weissman, J. Unpublished data.
2. Laws, E. Unpublished data.

Navicula sp.

Strain: NAVIC7 (BB 260)

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

Cells of *Navicula* sp. NAVIC7
(a) living cells; (b) cleared valves
Scale: (a) 1 cm = 10 μ m; (b) 1 cm = 5 μ m

Collection site: Biloxi Bridge, Mississippi, USA (M. Tadros)

Size: 6-7 μ m $\times$ 3-4 μ m

Growth form: unicellular

Growth rate at optimum (or maximum recorded): 3.8 doublings day⁻¹

Culture conditions:

Vitamins required:	not determined
Available nitrogen sources:	nitrate, urea, ammonia
Suitable media:	GPM
Nutritional modes:	autotrophic

Temperature/salinity growth responses:

Exponential growth rate (doublings day⁻¹) in batch culture.

A = seawater.

Chemical composition:

Growth Conditions	% lipid	% protein	% carbohydrate	Ref.	Basis
B	16	27	30	1	AFDW
B,N(N)	35	15	23	1	AFDW
B,N(Si)	43	15	18	1	AFDW

Taxonomic and morphological notes:

In the light microscope this strain is similar to three taxa: *Navicula pelliculosa*, *N. permitis*, or *N. saprophila*. Transmission electron microscopy is planned to determine positive species identification.

Nitzschia sp.

Strain: NITZS1

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

Cells of *Nitzschia* sp. NITZS1
(a) living cells; (b) cleared valve
Scale: (a) 1 cm = 10 μ m; (b) 1 cm = 5 μ m

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

Size: 40-53 μ m $\times$ 6-8 μ m

Growth form: unicellular

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 auxospores.

Outdoor culture history:

1. *Nitzschia* spp. have been noted to be occasional dominant algae in seawater-enrichment cultures in Woods Hole, Massachusetts (4), and in 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 protozoa (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 Meeting*. 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 Meeting*. 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. De Pauw, N., H. Verlet & L. De Leenheer. 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 (Kuetz.) Grunow

Strain: NITZS2 (DI-160)

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

Cells of *Nitzschia dissipata* NITZS2
(a) living cells; (b) cleared valve
Scale: (a) 1 cm = 10 μm ; (b) 1 cm = 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: unicellular

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 ⁻¹ 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.

Nitzschia communis Rabenhorst

Strain: NITZS14 (PV-2, ASU 0665)

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

Cells of *Nitzschia communis* NITZS14
(a) living cells; (b) cleared valves
Scale: (a) 1 cm = 10 μ m; (b) 1 cm = 5 μ m

Collection site: Palo Verde Irrigation District, Imperial County, California
(M. Sommerfeld)

Date: July 3, 1985
Water temperature: 34°C
Salinity: 10.87 mmho cm⁻¹ conductivity
pH: 8.3

Size: 22 μ m $\times$ 6 μ m

Growth form: unicellular

Growth rate at optimum (or maximum recorded): 1.41 doublings day⁻¹

Culture conditions:

Available nitrogen sources:	nitrate
Suitable media:	SERI Type II, 25 mmho cm ⁻¹
Temperature range:	26°C
Salinity range:	5-70 mmho cm ⁻¹
optimum:	10 mmho cm ⁻¹

Chemical composition:

Not determined, but lipid droplets visible with Nile Red.

Life cycle:

Vegetative division is the common reproductive method. The parental cell forms two new cells which are slightly smaller than the parental cell.

Oocystis pusilla Hansgirg

Strain: OOCYS1

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

Cells of *Oocystis pusilla* OOCYS1
Scale: 1 cm = 10 μ m

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 μ m $\times$ 8-10 μ m

Growth form: unicellular. 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 Meeting*. 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: PHAEO1 (TFX-1)

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

Cells of *Phaeodactylum tricornutum* PHAEO1
Scale: 1 cm = 10 μ m

Collection site: Woods Hole, Massachusetts, USA

Size: 15-22 μ m $\times$ 3-4 μ m

Growth form: unicellular

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 supplied as NH₄⁺ (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 have 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 $2\text{--}5 \text{ g C m}^{-2} \text{ d}^{-1}$.
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 $1\text{--}10 \text{ g DW m}^{-2} \text{ d}^{-1}$ (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 occurred (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 $1\text{--}6 \text{ g C m}^{-2} \text{ d}^{-1}$ (12,13).

Literature cited:

1. Terry, K.L. & J. Hirata. Unpublished data.
2. Terry, K.L., J. Hirata & E.A. Laws. 1983. Light-limited growth of two strains of the marine diatom *Phaeodactylum tricornutum* Bohlin: chemical composition, carbon partitioning and the diel periodicity of physiological processes. *J. Exp. Mar. Biol. Ecol.* 68:209-227.

3. Terry, K.L., J. Hirata & E.A. Laws. 1984. Light-, nitrogen-, and phosphorus-limited growth of *Phaeodactylum tricornutum* Bohlin strain TFX-1: chemical composition, carbon partitioning, and the diel periodicity of physiological processes. *J. Exp. Mar. Biol. Ecol.*, in press.
4. Cooksey, K.E. & B. Cooksey. 1974. Calcium deficiency can induce the transition from oval to fusiform cells in cultures of *Phaeodactylum tricornutum* Bohlin. *J. Phycol.* 10:89-90.
5. Raymont, J.E.G. & M.N.E. Adams. 1958. Studies on the mass culture of *Phaeodactylum*. *Limnol. Oceanogr.* 3:119-136.
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7. Ansell, A.D., J.E.G. Raymont & K.F. Lander. 1963. Studies on the mass culture of *Phaeodactylum*. III. Small-scale experiments. *Limnol. Oceanogr.* 8:207-213.
8. Ansell, A.D., J. Coughlin, K.F. Lander & L.A. Loosmore. 1963. Studies on the mass culture of *Phaeodactylum*. IV. Production and nutrient utilization in outdoor mass culture. *Limnol. Oceanogr.* 8:334-342.
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10. Guerrero Valero, S., T. Farina Tresquerras & A. Silva Abunin. 1981. Large-scale outdoor algal production for rearing seed oysters and clams to juvenile stage. In: *Nursery Culturing of Bivalve Molluscs*, Proc. Int. Workshop Nursery Cult. Bivalve Molluscs, Ghent, Belgium. C. Claus, N. de Pauw & E. Jaspers (eds.). pp. 117-139.
11. Braga, Y.Y. & L.D. Druehl. 1978. Seasonal growth and succession of tropical and introduced phytoplankton cultured in deep sea water. *Aquaculture* 14:1-12.
12. Mann, R. 1978. Growth of *Mytilus edulis* in a waste recycling aquaculture system. *Aquaculture* 13:351-354.
13. D'Elia, C.F., J.H. Ryther & T.M. Losordo. 1977. Productivity and nitrogen balance in large-scale phytoplankton cultures. *Water Res.* 11:1031-1040.

Phaeodactylum tricornutum Bohlin

Strain: PHAEO2 (BB)

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

Fusiform cells of *Phaeodactylum tricornutum* PHAEO2
Scale: 1 cm = 10 μ m

Source: W. Thomas, Scripps Institution

Size: fusiform cells = 15 μ m $\times$ 4 μ m

Growth form: unicellular, 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 NH₄⁺ (3,8).

Photoinhibition:

10% or more above $\sim 500 \mu\text{E m}^{-2} \text{s}^{-1}$.

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 PHAE02 differs significantly from PHAE01 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* PHAE01 for culture histories of other strains.)

1. A small ($\sim 0.5 \text{ m}^2$) shallow (2.2 cm) raceway system operated at Kaneohe, Hawaii, USA, in the mid 1970s gave a calculated production rate of $23 \text{ g AFDW m}^{-2} \text{ d}^{-1}$ (10).
2. *P. tricornutum* PHAE02 was grown in a shallow raceway system in Hawaii. Achieved production of $25 \text{ g m}^{-2} \text{ d}^{-1}$ (photosynthetic efficiency 5%-6%), but temperature control was required to achieve species survival (4,5,6,7).

Literature cited:

1. Thomas, W.H., D.L.R. Seibert, M. Alden, P. Eldridge, A. Neori & S. Gaines. 1981. Effects of light intensity and nitrogen deficiency on yield, proximate composition, and photosynthetic efficiency of *Phaeodactylum*. In: *Proceedings of the Subcontractors' Review Meeting, Aquatic Species Program*. Solar Energy Research Institute Publication SERI/CP-624-1228. pp. 33-58.
2. Fanunko, N. 1981. On the growth of marine diatom *Phaeodactylum tricornutum* in batch culture. *Acta Bot. Croat.* 40:111-116.
3. Terry, K. & J. Hirata. Unpublished data.
4. Laws, E.A. 1984. Optimization studies in shallow algal mass culture flumes. In: *Aquatic Species Program Review: Proceedings of the April 1984 Principal Investigators Meeting*. Solar Energy Research Institute Publication SERI/CP-231-2341. pp. 108-131.
5. Laws, E.A. 1983. Shallow algal mass culture systems. In: *Aquatic Species Program Review: Proceedings of the March 1983 Principal Investigators Meeting*. Solar Energy Research Institute Publication SERI/CP-231-1946. pp. 33-48.

6. Laws, E.A. 1981. Year one progress report on algal production raceway system. In: *Proceedings of the Subcontractors' Review Meeting, Aquatic Species Program*. Solar Energy Research Institute Publication SERI/CP-624-1228. pp. 1-31.
7. Laws, E.A., K.L. Terry, J. Wickman & M.S. Chalup. 1983. A simple algal production system designed to utilize the flashing light effect. *Biotechnol. Bioeng.* 25:2319-2336.
8. Terry, K.L., J. Hirata & E.A. Laws. 1983. Light-limited growth of 2 strains of the marine diatom *Phaeodactylum tricornutum*: chemical composition, carbon partitioning and the diel periodicity of physiological processes. *J. Exp. Mar. Biol. Ecol.* 68:209-228.
9. Cooksey, K.E. & B. Cooksey. 1974. Calcium deficiency can induce the transition from oval to fusiform cells in cultures of *Phaeodactylum tricornutum* Bohlin. *J. Phycol.* 10:89-90.
10. Raymond, L.P. 1978. Initial Investigations of a Shallow-Layer Algal Production System. Hawaii Natural Energy Inst. Tech. Report 7.

Pleurochrysis sp.

Strain: PLEUR1

Taxonomy: Division: Chrysophyta
 Class: Prymnesiophyceae
 Order: Isochrysidales
 Family: Hymenomonadaceae

Cells of *Pleurochrysis* sp. PLEUR1

(a) coccolith-bearing cell; (b) haploid swarmer; (c) pseudofilamentous stage

Scale: (a,c) 1 cm = 10 μ m; (b) 1 cm = 5 μ m

Collection site: Bitter Lakes Wildlife Refuge, New Mexico, USA (R. McIntosh, W. Barclay)

Date: May 7, 1985
Water temperature: 25°C
Salinity: 20 mmho cm⁻¹
pH: 7.6

Size: 9-14 μ m wide $\times$ 9-15 μ m long

Growth form: unicellular flagellate or pseudofilamentous benthic stage

Growth rate at optimum (or maximum recorded): 1.5 doublings day⁻¹

Culture conditions:

Vitamins required:	not determined
Available nitrogen sources:	urea, nitrate
Suitable media:	GPM
Nutritional modes:	autotrophic
Temperature range:	>10° to 33°C
optimum:	25°C
Salinity range:	<10 to >70 mmho cm ⁻¹
optimum:	25 mmho cm ⁻¹

Temperature/salinity growth responses:

Exponential growth rate (doublings day⁻¹) in batch culture.

A = SERI Type I inland saline water;

B = artificial seawater.

Physiological notes:

Goes into pseudofilamentous resting stage when calcium concentration is low in culture medium.

Taxonomic notes:

This species is soon to be described under the name *Pleurochrysis dentata*.

Life cycle:

Heteromorphic alternation of generations. Haploid stages are devoid of coccoliths; diploid stages bear coccoliths. Flagellated and benthic stages occur in both diploids and haploids.

Tetraselmis suecia

Strain: TETRA1 (S/PLATY-1)

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

Cells of *Tetraselmis suecia* TETRA1
Scale: 1 cm = 10 μ m

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

Date: Summer 1983

Size: 13-18 μ m $\times$ 13 μ m

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: SERI Type I, 10 mmho cm⁻¹

Nutritional modes: autotrophic

Temperature range: not determined

optimum: 34°C (2)

Salinity range: 15 to >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 $\approx$ 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 m² raceway system. High productivity (35-45 g m⁻²d⁻¹) at a salinity of 15-30 o/oo and at 28°-32°C (1,2).

Literature cited:

1. 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.
2. Laws, E.A. 1984. Optimization studies in shallow algal mass culture flumes. In: *Aquatic Species Program Review: Proceedings of the April 1984 Principal Investigators Meeting*. Solar Energy Research Institute Publication SERI/CP-231-2341. pp. 108-132.
3. Trick, C.G. 1979. The life cycle of *Platymonas impellucida* Prasinophyceae in culture. *Proc. N.S. Inst. Sci.* 29(2):215-222.

Tetraselmis sp.

Strain: TETRA4

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

Cells of *Tetraselmis* sp. TETRA4
(a) Flagellated cell with apical pit on left; (b) dividing cells
Scale: 1 cm = 10 μ m

Collection site: Stock Island, Florida Keys, Florida, USA (R. Carlson)

Date: November 10, 1983
Salinity: 10 ppt

Size: 10-12 μ m $\times$ 6-8 μ m

Growth form: unicellular

Growth rate at optimum (or maximum recorded): 2.17 doublings day⁻¹

Culture conditions:

Vitamins required:	not determined
Available nitrogen sources:	nitrate
Suitable media:	f/2
Nutritional modes:	photoautotrophic
Temperature range:	15°-36°C
optimum:	29°C
Salinity range:	8-60 mmho cm ⁻¹
optimum:	28 mmho cm ⁻¹

Temperature/salinity growth responses:

Exponential growth rate (doublings day⁻¹) in batch culture:

Water Type	Growth (doublings day ⁻¹)
Seawater, 25 mmho cm ⁻¹	1.5
Seawater, 40 mmho cm ⁻¹	0.7
SERI Type I, 25 mmho cm ⁻¹	1.2
SERI Type I, 40 mmho cm ⁻¹	1.1
SERI Type II, 25 mmho cm ⁻¹	1.4
SERI Type II, 40 mmho cm ⁻¹	1.1

Growth response in batch culture of *Tetraselmis* sp. to temperature and salinity.
Contours are exponential growth rate (doublings day⁻¹).

Chemical composition:

Growth Conditions	% lipid	% protein	% carbohydrate	Ref.	Basis
B	20.5	7.3	48.6		AFDW
B,N(N)	22.3	3.5	36.5		AFDW

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 (2).

Outdoor culture history:

Tetraselmis sp. was grown in full-strength seawater in 1.4 m² ponds in California. Achieved production of 17 g m² d⁻¹ (photosynthetic efficiency = 4.4%) over a period of 25 days.

Literature cited:

1. Weissman, J. Unpublished data.
2. Trick, C.G. 1979. The life cycle of *Platymonas impellucida* Prasinophyceae in culture. *Proc. N.S. Inst. Sci.* 29(2):215-222.

Thalassiosira weissflogii (Grun.) Fryx. and Hasle

Strain: THALA2

Taxonomy: Division: Chrysophyta
 Class: Bacillariophyceae
 Order: Centrales
 Family: Thalassiosiraceae

Cells of *T. weissflogii* THALA2
(a) living cell; (b) cleared valves
Scale: (a) 1 cm = 10 μm ; (b) 1 cm = 5 μm

Collection site: Warm Spring, Colorado River at Glenwood Springs, Colorado, USA
(W. Barclay)

 Date: September 6, 1985
Water temperature: 27°C
 Salinity: 12 mmho cm^{-1}
 pH: 7.8

Size: 12-24 μm diameter

Growth form: unicellular

Growth rate at optimum: 1.39 doublings day^{-1}

Culture conditions:

Vitamins required:	not determined
Available nitrogen sources:	urea
Suitable media:	SERI Type I, 10 mmho cm ⁻¹ ; GPM, 25 mmho cm ⁻¹
Nutritional modes:	autotrophic
Temperature range:	10°-30°C
optimum:	25°-30°C
Salinity range:	10-70 mmho cm ⁻¹
optimum:	10-40 mmho cm ⁻¹

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.

Thalassiosira weissflogii (Grun.) Fryx. and Hasle

Strain: THALA6 (SH14-89)

Taxonomy: Division: Chrysophyta
Class: Bacillariophyceae
Order: Centrales
Family: Thalassiosiraceae

Cells of *T. weissflogii* THALA6
(a) living cell; (b) cleared valves
Scale: (a) 1 cm = 10 μ m; (b) 1 cm = 5 μ m

Collection site: Menehune Fishpond, Kauai, Hawaii, USA 21° 57' 05.4" N, 159° 22' 39.6" W (R. York).

Date: August 7, 1985
Water temperature: 29.2°C
Salinity: 17.8 mmho cm⁻¹
pH: 7.5
Dissolved oxygen: 5.8 ppm

Size: 10–15 μm $\times$ 10–15 μm

Growth form: unicellular

Growth rate (maximum recorded): 1.43 doublings day⁻¹

Production (maximum recorded): 33 g DW m⁻² d⁻¹

Culture conditions:

Vitamins required: not determined

Available nitrogen sources: ammonium, nitrate

Suitable media: f/2, GPM

Nutritional modes: autotrophic

Temperature range: 17.7°-42.8°C

Salinity range: 17.8-45.0 mmho cm⁻¹

Appendix

CULTURE MEDIA

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ASW Medium
(Darley and Volcani, 1969)

To 1 L of distilled water add:

NaCl	23.6 g
MgSO ₄ ·7H ₂ O	4.9 g
MgCl ₂ ·6H ₂ O	4.1 g
CaCl ₂	1.1 g
KCl	75 mg
KNO ₃	303 mg
Na ₂ EDTA	12 mg
Na ₂ SiO ₃ ·9H ₂ O	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 K₂HPO₄ in 100 mL distilled water, and add 10 mL/L at time of inoculation.

Trace element stock (for 1 L):

H ₃ BO ₃	0.568 g
ZnCl ₂	0.624 g
CuCl ₂ ·2H ₂ O	0.268 g
Na ₂ MoO ₄ ·2H ₂ O	0.252 g
CoCl ₂ ·6H ₂ O	0.42 g
FeSO ₄	1.36 g
MnCl ₂ ·4H ₂ O	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:

<u>Salt</u>	<u>Grams</u>
NaNO ₃	10.0 g
CaCl ₂ ·2H ₂ O	1.0 g
MgSO ₄ ·7H ₂ O	3.0 g
K ₂ HPO ₄	3.0 g
KH ₂ PO 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 H₂O (or 50 g Na₂EDTA dissolved in 1 L distilled H₂O).
2. 4.98 g FeSO₄·7H₂O dissolved in 1 L of acidified water (acidified H₂O: 1.0 mL H₂SO₄ dissolved in 1 L distilled H₂O).
3. 11.42 g H₃BO₃ dissolved in 1 L distilled H₂O.
4. The following, in amounts indicated, all dissolved in 1 L distilled water:
ZnSO₄·7H₂O, 8.82 g; MnCl₂·4H₂O, 1.44 g; MoO₃, 0.71 g; CuSO₄·5H₂O, 1.57 g;
Co(NO₃)₂·6H₂O, 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 ₂ SiO ₃ · 9H ₂ O	28 mg
H ₃ BO ₃	6 mg
FeCl ₃	0.4 mg
Na ₂ EDTA	11 mg
Tris	2420 mg
KNO ₃	505 mg
KH ₂ PO ₄	54 mg
Vitamin B ₁₂	1.0 µg
Thiamine-HCl	0.2 µg
Biotin	1.0 µg
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 ₂ PO ₄ ·H ₂ O	5 mg
Na ₂ SiO ₃ ·9H ₂ O	30 mg
Thiamine-HCl	100 µg
Biotin	0.5 µg
B ₁₂	0.5 µg
Trace elements stock solution	1 mL

Trace elements stock solution (for 1 L):

Na ₂ EDTA	4.36 g
FeCl ₃ ·6H ₂ O	3.15 g
MnCl ₂ ·4H ₂ O	180 mg
CuSO ₄ ·5H ₂ O	10 mg
ZnSO ₄ ·7H ₂ O	22 mg
CoCl ₂ ·6H ₂ O	10 mg
NaMoO ₄ ·2H ₂ O	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 NaHCO₃ 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 ₂ HPO 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 K₂HPO₄ addition separately in 10 mL of distilled water and add after the medium cools.

PII trace element stock (for 1 L):

Na ₂ EDTA	6.0 g
FeCl ₃ ·6H ₂ O	0.29 g
H ₃ BO ₃	6.84 g
MnCl ₂ ·4H ₂ O	0.86 g
ZnCl ₂	0.06 g
CoCl ₂ ·6H ₂ O	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 filter 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 NaHCO₃ should also be added to the medium. Additionally, 250-500 mg L⁻¹ NaSiO₃·9H₂O 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 ₂ HPO ₄	20 mg
MgSO ₄ ·H ₂ O	100 mg
CaCl ₂ ·6H ₂ O	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 ₂ CO ₃	25.44 g
NaHCO ₃	15.12 g
Na ₂ SO ₄	14.20 g
KCl	2.91 g
H ₃ BO ₃	1.92 g
KNO ₃	1.01 g
MgSO ₄	35 mg
Na ₂ SiO ₃	198 mg
Ca(NO ₃) ₂	70 mg
KH ₂ PO 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 ·7H ₂ O	84 mg
H ₃ BO ₃	600 mg
CoCl ₂ ·6H ₂ O	150 mg
CuSO ₄	37 mg
MnCl ₂ ·4H ₂ O	400 mg
(NH ₄) ₆ Mo ₇ O ₂₄ ·4H ₂ O	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 ·6H ₂ O	508 mg
Na ₂ CO ₃	392 mg
CaCl ₂	28 mg
KCl	246 mg
Na ₂ SO ₄	207 mg
Na ₂ B ₄ O ₇ ·10H ₂ O	9 mg
NaF	11 mg
NaNO ₃	849 mg
KH ₂ PO 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.

Salt	Conductivity (mmho cm^{-1})				
	10	25	40	55	70
CaCl_2	0	3,932	5,618	7,610	8,430
$\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$	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* (0.6M N)	1 mL
KH_2PO_4 (0.6M)	1 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 .

$250\text{--}500 \text{ mg L}^{-1} \text{ Na}_2\text{SiO}_3 \cdot 9\text{H}_2\text{O}$ 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.

Salt	Conductivity (mmho cm^{-1})				
	10	25	40	55	70
CaCl_2	28	28	28	28	28
$\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$	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
NaCl	1,511	8,078	12,963	20,588	26,075

Suggested enrichments (mL/L) are:

Nitrogen source* (0.6M N)	1 mL
KH_2PO_4 (0.6M)	1 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 .

250–500 mg L^{-1} $\text{Na}_2\text{SiO}_3 \cdot 9\text{H}_2\text{O}$ 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 ₂ CO ₃	1.322 g
Na ₂ SO ₄	3.392 g
CaCl ₂	28 mg
MgSO ₄ ·7H ₂ O	790 mg
KCl	430 mg
Na ₂ B ₄ O ₇ ·10H ₂ O	169 mg
NaF	9 mg
NaNO ₃	849 mg
KH ₂ PO 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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