SERI/SP-231-2486
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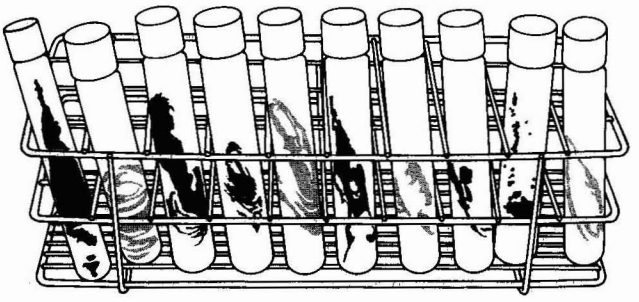

Microalgae Culture Collection 1984-1985

September 1984

Prepared by the
Microalgal Technology Research Group

Solar Energy Research Institute
A Division of Midwest Research Institute

1617 Cole Boulevard
Golden, Colorado 80401

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INTRODUCTION

Many of the advances in phycology during the last decade in the areas of taxonomy, cytology, genetics, biochemistry, and physiology have been made through the use of recognized clones or strains of algae maintained and distributed by culture collections. The Microalgae Culture Collection at the Solar Energy Research Institute has been established for the maintenance and distribution of strains that have been characterized for biomass fuel applications. This collection provides documented reference clones or strains of microalgae to researchers and organizations interested in conducting biological or biofuels research or in developing microalgal biomass technologies. Culture and composition data have been compiled on each strain maintained in the collection.

Microalgal research at SERI focuses on biomass energy production: thus, the SERI culture collection will be 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. A set of criteria has been established to guide the selection of clones or strains for the collection. These criteria, in descending order of importance, are as follows:

- Energy yield (growth rate x energy content)
- Type of fuel products available from biomass (hydrocarbon, diesel, alcohol, methanol)
- Environmental tolerance range (temperature, salinity, pH)
- Performance in mass culture (highly competitive, predator resistant)
- Media supplementation requirements (addition of vitamins, trace minerals)
- Amount of culture and composition data available on the clone or strain
- Budget for the culture collection.

A steering committee is convened once a year to review both potential new clones or strains for addition into the collection and existing clones or strains in the collection for possible deletion.

The culture collection at SERI currently contains 12 strains of microalgae. Included are 5 strains of Chlorophyta, *Ankistrodesmus*, *Oocystis*, *Chlorella*, *Platymonas*, and *Botryococcus*; and 7 strains of Chrysophyta, *Nannochloropsis*, *Phaeodactylum* (2), *Nitzschia*, *Chaetoceros*, and *Isochrysis* (2). Researchers may also request abstracts of previous work published concerning a particular strain. Where there has been little work published, references for other strains or species of the same genus are included. These abstracts are maintained in a data base that may be referenced using pertinent key words. This data base is updated four times a year.

Explanatory Notes

A major emphasis of the SERI culture collection is to make available most of the existing scientific and culture data for each strain in the collection. Each strain is listed in the catalog with a summary of its most important physiological and culture characteristics.

Although most of the data listed in the summary sheets is 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	continuous culture
	SC	semicontinuous culture
	MC	outdoor mass culture

	N(X)	nutrient limited, where X is replaced by P for phosphorus limitation, N for nitrogen limitation, or C for carbon (CO_2) limitation
	L(n)	light limited, where n is the culture irradiance in μEinst m^{-2} s^{-1}
Basis:	C	carbon
	DW	dry weight
	AFDW	ash-free dry weight

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

Fuel Options. Each of the three biochemical fractions (lipids, carbohydrates, and proteins) can be converted into fuels. Lipids, with the highest energy content of the three, can be converted into a fuel similar to diesel oil by the process of transesterification. Carbohydrates are commonly converted to ethanol by fermentation. Alternatively, all three fractions can be converted to methane gas by anaerobic digestion. Fuel production options were calculated for each strain based on its chemical composition under nutrient limited conditions. The assumptions and procedures for these calculations have been outlined in *Fuel Options from Microalgae with Representative Chemical Compositions* (by 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.

References. Computer searches of the scientific literature have been conducted for each genus in the collection, for the following information: (1) physiology; (2) ecology; (3) culture; (4) chemical composition; (5) taxonomy; (6) ultrastructure; and (7) food for higher organisms. These computer searches cover articles published during the past five years. The number of references with abstracts available in each category is summarized in the reference section of each strain's summary sheet. Copies of these references and abstracts are available on request with each strain of algae.

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

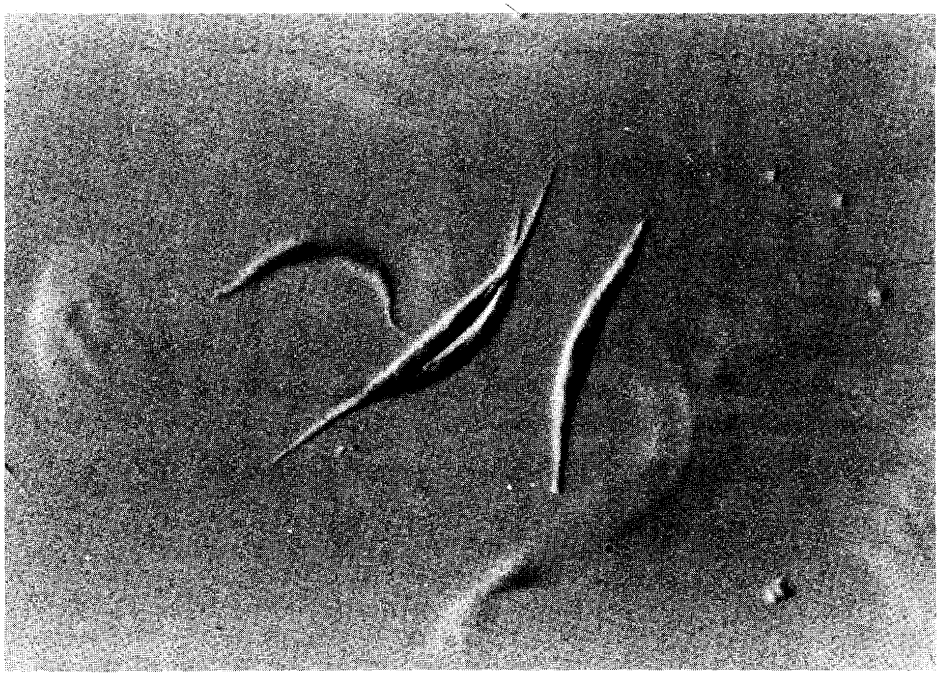
Cultures will usually be sent within one week of receipt of a request. Questions about the culture collection or requests for information can be made by phone to (303)231-1842. References and abstracts on each genus maintained in the collection can be requested for each strain. Refer to the strain summary sheets for pertinent categories and numbers of references in each.

We request that investigators using species from this collection please send us copies of publications resulting from research on these strains so that the species data bases can be continually updated.

Ankistrodesmus falcatus

Strain: Pyramid Lake (S/ANKIS-1)

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



Ankistrodesmus falcatus cells (Scale: 1 cm = 10 μm)

Collection site: Pyramid Lake, Nevada, USA (1)

Date: October 1982
Water Temperature: 17°C
Salinity: 5 o/oo
pH: 9.1

Size: 35-57 μm x 3μm

Growth form: unicells

Growth rate at optimum (or maximum recorded): 1.06 day^{-1} (20°C)* (2)

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

Culture conditions:

Vitamins required: None

Available nitrogen sources: Urea, Nitrate, Ammonium (Urea gives more rapid growth than nitrate) (1)

Suitable media: Pyramid Lake

Nutritional modes: Photoautrophic (*A. angustus* heterotrophic (3))
(*A. fusiformis* heterotrophic (4))

Temperature range: $18^{o}C$ - $31^{o}C$ (1)

optimum: $26^{o}C$ (1)

Salinity range: 1 o/oo - 10 o/oo (1)

optimum: 7 o/oo (1)

Chemical composition:

Growth conditions	% lipid	% protein	% carbohydrate	Ref.	Basis
B	27.2	34.0	11.4	5	DW
B, N(N)	40.3	14.4	18.3	5	DW
B, N(C)	19.5	28.6	9.2	5	DW

Lipid composition: (6)

Growth Conditions	Hexane	Benzene	Chloroform	Acetone	Methanol
B	---	2.5	9.8	72.6	19.5
B, N(N)	---	3.3	13.5	66.5	12.1
B, N(C)	---	5.8	14.1	66.8	10.5

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	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. pH 3 is lethal, slight growth at pH 4, optimum at pH 6 for *Ankistrodesmus* sp. (7)*
2. N:P requirement ratio is ~21 (mol:mol) (8)*
3. Many strains of *Ankistrodesmus* have low salt tolerance (<10 o/oo) (9)*
4. Ash 7-14% of dry weight (5)

Life cycle:

Reproduction is by division of cell into two, four, or eight autospores. Vegetative cells can also form resting cells (aplanospores). (10)

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. (6)
2. An unspecified species of *Ankistrodesmus* has been cultured in South Africa for the removal of nitrogen from industrial wastes. (11)
3. *Ankistrodesmus* sp. was a component of a population grown on diluted pig slurry (liquid phase) in a Dortmund-type system in Northern Ireland. (12)

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, produced 8-10 g m^{-2} d^{-1}. (13)

References (number available in each category):

Physiology:	81
Ecology:	59
Culture:	13
Chemical composition:	6
Taxonomy:	7
Ultrastructure:	2
Food for higher organisms:	5

Literature cited:

1. Thomas, W.H., D.L.R. Seibert, M. Alden & P. Eldridge. 1984. Cultural requirements, yields, and light utilization efficiencies of some desert saline microalgae. In: *Aquatic Species Program Review: Proceedings of the April 1984 Principal Investigators Meetings*. Solar Energy Research Institute, Publ. 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. Conner, A.J. 1981. The differential sensitivity of phytoplankton to polychlorinated biphenyls when cultured heterotrophically and photoautotrophically. *Environ. Exp. Bot.* 21:241-248.

4. Vilicic, D. 1979. On heterotrophic nutrition of some species of green algae. *Acta. Bot. Croat.* 38:45-54.

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

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

7. Baker, M.D., C.I. Mayfield, W.E. Ihrniss & P.T.S. Wong. 1983. Toxicity of pH, heavy metals and bisulfite to a fresh water green alga *Ankistrodesmus* sp. *Chemosphere*. 12:35-44.

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

9. 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.

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

11. 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.

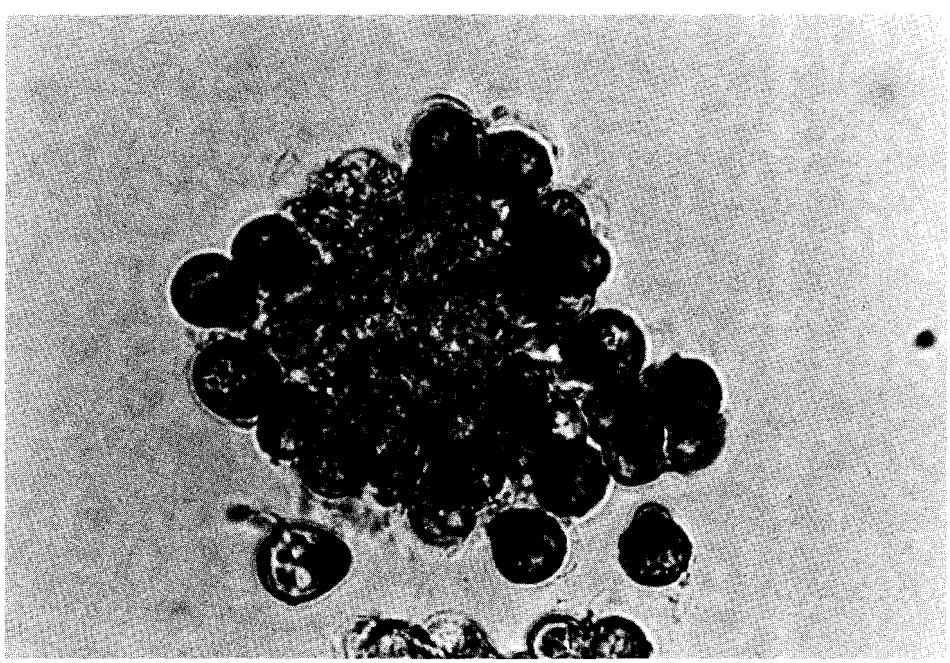
12. Fallowfield, H.J. & M.K. Garrett. 1983. Mass outdoor culture of algae on effluents in Northern Ireland. *Br. Phycol. J.* 18:203.

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

Botryococcus braunii Kutz

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

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



Colony of *Botryococcus braunii*. (Scale: 1 cm = 10 μm)

Source: Univ. of Texas culture collection

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

Growth form: colonial

Growth rate at optimum (or maximum recorded): 0.37 day^{-1}

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	14.0	2	DW
B, N(N)	54.2	20.6	14.3	2	DW

Lipid composition: (1)

Growth conditions	Hexane	Benzene	Chloroform	Acetone	Methanol
B	4.6	51.4	4.5	30.0	9.4
B, N(N)	14.9	52.7	3.4	21.6	7.4

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	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 0.5M 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:

1. 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)

References (number available in each category):

Physiology:	6
Ecology:	21
Culture:	6
Chemical composition:	18
Taxonomy:	0
Ultrastructure:	6
Food for higher organisms:	0

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. Tornabene, T., A. Ben-Amotz, S. Raziuddin & J. Hubbard. 1984. Chemical profiles of microalgae with emphasis on lipids. In: *Screening for lipid yielding microalgae: activities for 1983. Final Subcontract Report.* Solar Energy Research Institute, Publ. SERI/STR-231-2207. pp. 16-38.

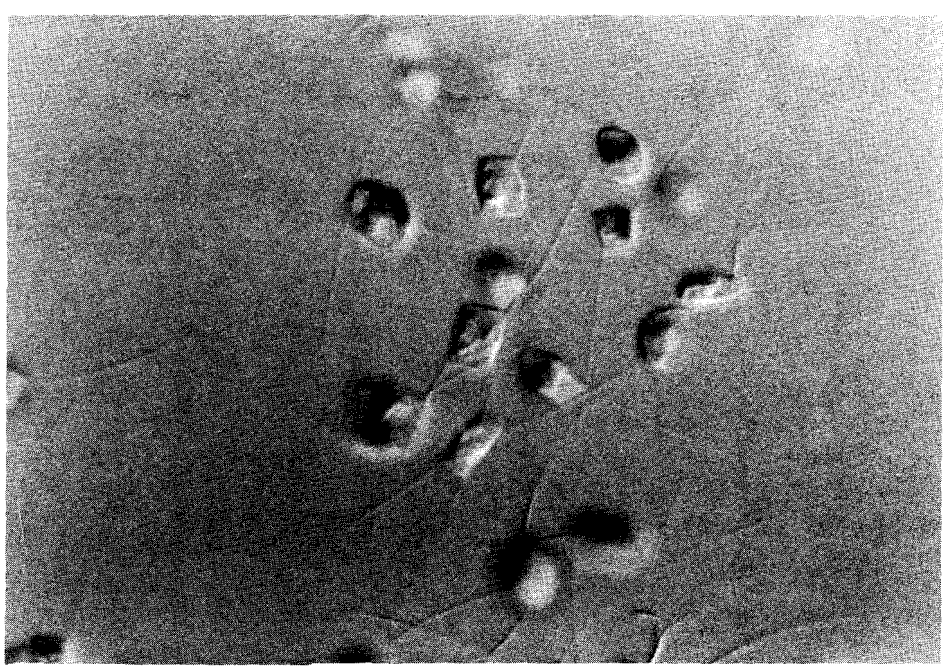
3. Tornabene, T.G. 1984. Chemical profile of microalgae with emphasis on lipid. In: *Aquatic Species Program Review: Proceedings of the April 1984 Principal Investigators Meetings.* Solar Energy Research Institute, Publ. SERI/CP-231-2341. pp. 64-78.

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

Chaetoceros gracilis Schutt

Strain: (S/CHAET-1)

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



Chaetoceros gracilis cells. (Scale: 1 cm = 10 μm)

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

Size: 5-7 μm x 4 μm (setae = 30-37 μm)

Growth form: Unicells, chains

Growth rate at optimum (or maximum recorded): 2.25 day^{-1} (1)

Culture conditions:

Vitamins required: None (2)

Available nitrogen sources: ammonium, nitrate

Suitable media: GPM

Nutritional modes: autotrophic

Temperature range: not determined

optimum: 28-30°C (3)

Salinity range: 15 o/oo - 35 o/oo

optimum: not determined

Chemical composition:

Growth conditions	% lipid	% protein	% carbohydrate	Ref.	Basis
MC	20.5	48.6	-----	4	DW

Physiological notes:

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

Life cycle:

Vegetative cell division is the ordinary method of reproduction. Sexual reproduction is oogamous resulting in 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 using artificial upwelling at Seward, AK, USA. (7)

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

References (number available in each category):

Physiology:	83
Ecology:	150
Culture:	25
Chemical composition:	14
Taxonomy:	5
Ultrastructure:	2
Food for higher organisms:	49

Literature cited:

1. Thomas, W.H. & A.N. Dodson. 1968. Effects of phosphate concentration on cell division rates and yield of a tropical oceanic diatom. *Biol. Bull.* 134:199-208.

2. de Pauw, N., J. Verbonen & C. Claus. 1983. Large-scale microalgae production for nursery rearing of marine bivalves. *Aquacult. Engr.* 2:27-47.

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

4. Hirata, J. Personal communication.

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

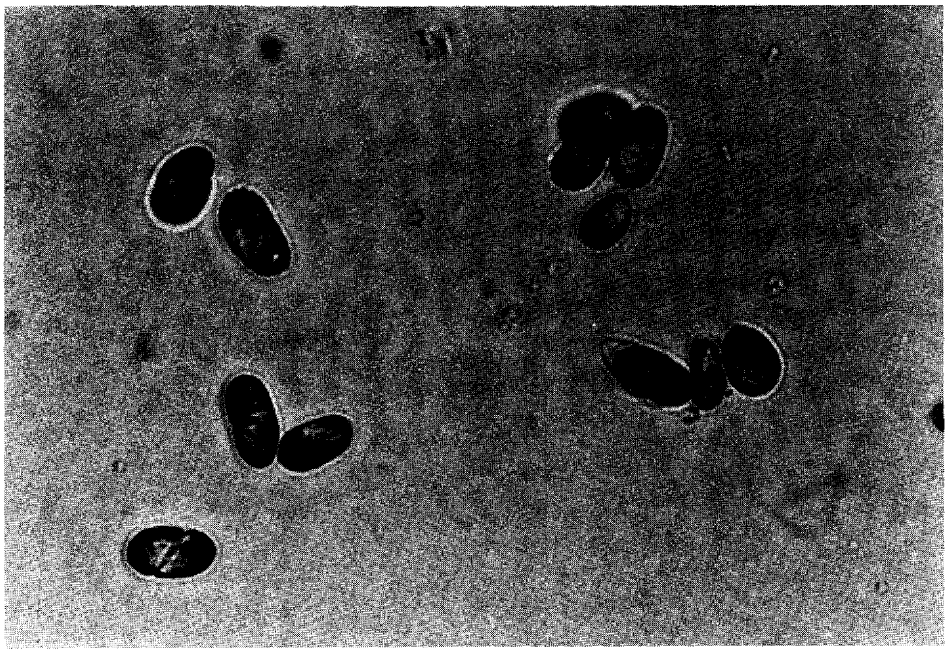
6. Roden, C.M. & K.W. O'Mahony. 1984. Competition as a mechanism of adaptation to environmental stress in outdoor cultures of marine diatoms. *Mar. Ecol. Prog. Ser.* 16:219-228.

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

Chlorella sp.

Strain: S01 (S/CHLOR-1)

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



Cells of *Chlorella* sp. (S01). (Scale: 1 cm = 10 μm)

Collection site: Construction ditch, Golden, Colorado

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

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

Growth form: unicells

Growth rate at optimum (or maximum recorded): 0.92 day^{-1}

Chlorella sp.

Culture conditions:

Vitamins required: none

Available nitrogen sources: nitrate, ammonium, urea

Suitable media: Bolds Basal

Nutritional modes: autotrophic

Temperature range: 15-39°C (1)

optimum: 35°C (1)

Salinity range: 0-18 o/oo (1)

optimum: 2-3 o/oo (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 that are freed by rupture of the parental cell wall.

Outdoor culture history:

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

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

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

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

References (number available in each category):

Physiology:	313
Ecology:	48
Culture:	31
Chemical composition:	47
Taxonomy:	6
Ultrastructure:	3
Food for higher organisms:	39

Literature cited:

1. Lien, S. 1984. Unpublished data.

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

3. Lien, S. & K.G. Spencer. 1984. Physiology of oil producing microalgae in response to stress conditions. In: *Aquatic Species Program Review: Proceedings of the April 1984 Principal Investigators Meetings.* Solar Energy Research Institute, Publ. 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.

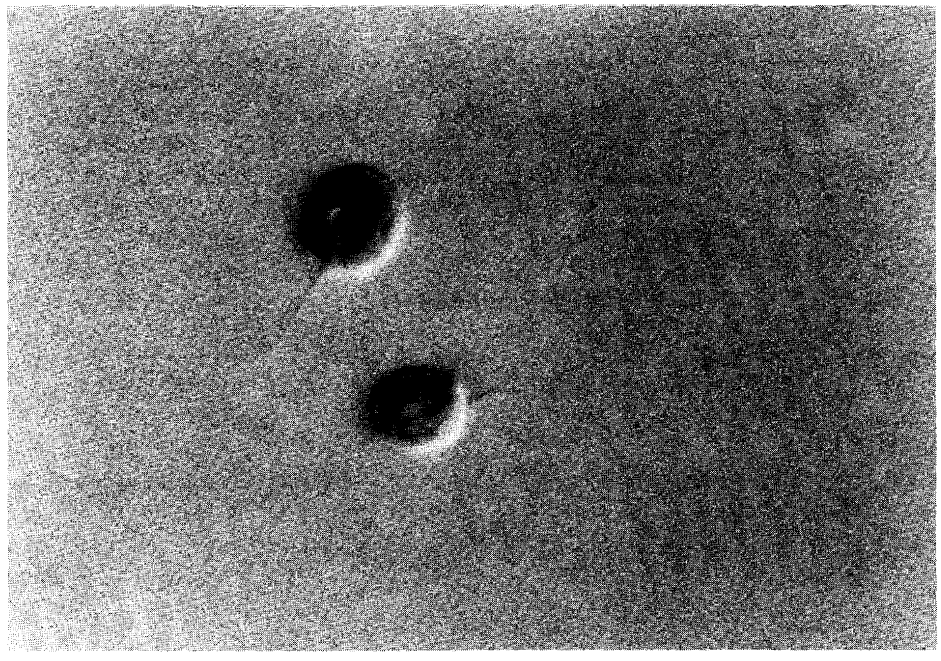
5. Lincoln, A.P. & D.T. Hill. 1980. An integrated microalgae system. In: *Algae Biomass.* Shelef, G. & C.J. Soeder, (eds.). Elsevier/North-Holland Biomedical Press. pp. 229-244.

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.

Isochrysis aff. *galbana* Green

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

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



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

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

Size: 7-4 μm x 4 μm

Growth form: flagellated unicells

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

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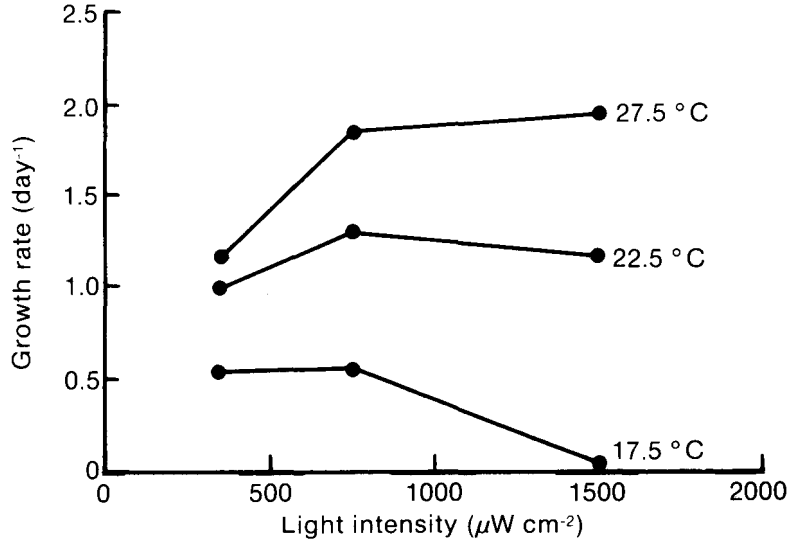
*Recently, a strain of *Isochrysis* sp. isolated in Israel has been added to the SERI collection. In many respects, this strain performs similarly to the Tahitian strain. Some data collected for this strain are presented here with an asterisk. Either or both strains may be ordered.

Culture conditions:

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

Light curve of growth:



after (1)

Chemical composition:

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

Lipid composition:

Growth Conditions	Hexane	Benzene	Chloroform	Acetone	Methanol	Ref.
SC?	1.4	27.4	32.1	26.5	12.6	
SC?, N(N)	2.2	28.4	18.0	26.1	25.3	
SC*	1.5	15.2	13.5	31.6	38.2	3
SC, N(N)*	2.5	35.6	12.7	28.0	21.2	3

Fuel production options:

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

Total energy content: 18.9 MJ/kg dry weight

Physiological notes:

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

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

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

Life cycle:

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

Outdoor culture history:

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

References (number available in each category):

Physiology:	25
Ecology:	15
Culture:	11
Chemical composition:	7
Taxonomy:	0
Ultrastructure:	0
Food for higher organisms:	86

Literature cited:

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

2. Richmond, A. 1984. Development of outdoor system for production of lipid rich halotolerant microalgae. In: *Aquatic Species Program Review: Proceedings of the April 1984 Principal Investigators Meetings*. Solar Energy Research Institute, Publ. SERI/CP-231-2341. pp. 195-205.

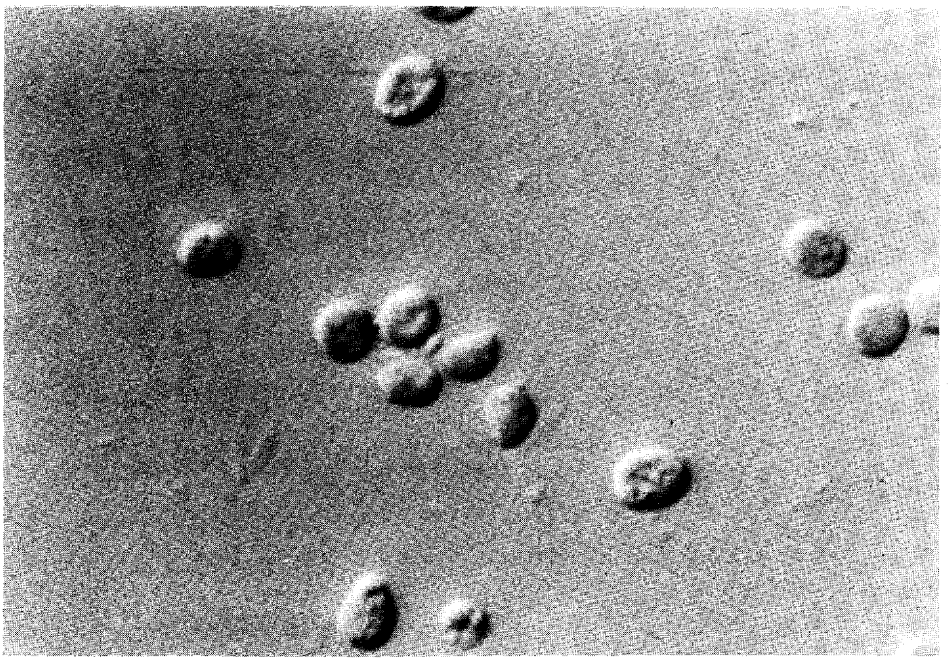
3. Ben-Amotz, A. 1984. Development of outdoor raceway capable of yielding halotolerant microalgae, identification of oil-rich strains. In: *Aquatic Species Program Review: Proceedings of the April 1984 Principal Investigators Meetings*. Solar Energy Research Institute, Publ. SERI/CP-231-2341. pp. 186-194.

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: Avault, J.W., Jr. *Proceedings of the Eleventh Annual Meeting, World Mariculture Society*. pp. 192-201.

Nannochloropsis salina Hibberd

Strain: GSBSTICHO (S/NANNO-1)

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



Nannochloropsis salina cells. (Scale: 1 cm = 5 μm)

Collection site: Great South Bay, Long Island, NY

Date: 1952 (John Ryther)

Size: 2.5-5 μm x 1.5-1.7 μm

Growth form: unicellular

Growth rate at optimum (or maximum recorded): 0.73 day^{-1}

Culture conditions:

Vitamins required: not determined

Available nitrogen sources: ammonium, urea, nitrate

Suitable media: f/2

Nutritional modes: autotrophic

Temperature range: 17-32°C (3)

optimum: 28°C (3)

Salinity range: 6 o/oo - 60 o/oo (3)

optimum: 30 o/oo (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: (5)

Growth conditions	Hexane	Benzene	Chloroform	Acetone	Methanol
B	2.5	12.4	25.4	28.7	31
B, N(N)	4.0	40.2	35.5	16.0	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	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:

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

References (number available in each category):

Physiology:	4
Ecology:	1
Culture:	1
Chemical composition:	4
Taxonomy:	1
Ultrastructure:	0
Food for higher organisms:	1

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. Linn. Soc.* 82:93-119

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

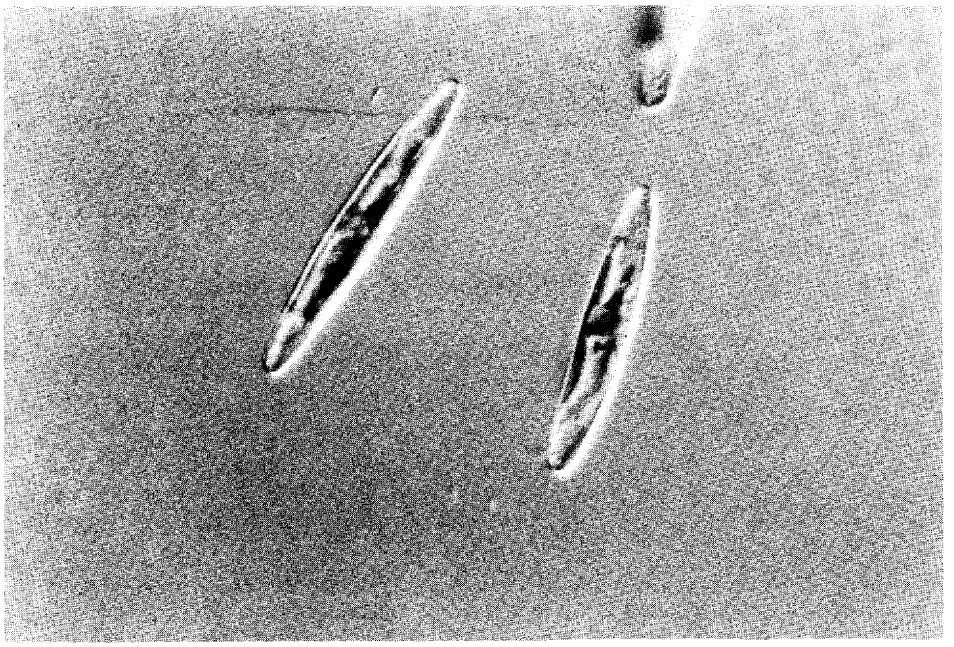
4. Tornabene, T.G. 1984. Chemical profile of microalgae with emphasis on lipids. In: *Aquatic Species Program Review: Proceedings of the April 1984 Principal Investigators Meetings*. Solar Energy Research Institute, Publ. SERI/CP-231-2341. pp. 64-79.

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

Nitzschia sp.

Strain: Mono Lake (S/NITZS-1)

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



Mono Lake *Nitzschia* sp. cells (Scale: 1 cm = 10 μm)

Collection site: Mono Lake, California (by David Chapman, UCLA)

Size: 40-53 μm x 6-8 μm

Growth form: unicells

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

Nitzschia sp.

Culture conditions:

Vitamins required: none

Available nitrogen sources: nitrate (best), urea

Suitable media: Mono Lake

Nutritional modes: autotrophic

Temperature range: 10-44°C (1)

optimum: 30-36°C (1)

Salinity range: 30 o/oo - 90 o/oo (1)

optimum: 50 o/oo - 70 o/oo (1)

Chemical composition:

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

Lipid composition: (2)

Growth conditions	Hexane	Benzene	Chloroform	Acetone	Methanol
B	0.9	1.7	51.2	22	24.6

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:

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

Life cycle:

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

Outdoor culture history:

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

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

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

References (number available in each category):

Physiology:	3
Ecology:	20
Culture:	4
Chemical composition:	1
Taxonomy:	1
Ultrastructure:	2
Food for higher organisms:	2

Literature cited:

1. Thomas, W.H., D.L.R. Seibert, M. Alden, P. Eldridge, A. Neori & S. Gaines. 1983. Selection of high-yielding microalgae from desert saline environments. In: *Aquatic Species Program Review: Proceedings of the March 1983 Principal Investigators Meetings.* Solar Energy Research Institute, Publ. 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, Publ. SERI/CP-231-1946. pp. 123-135.

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

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

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

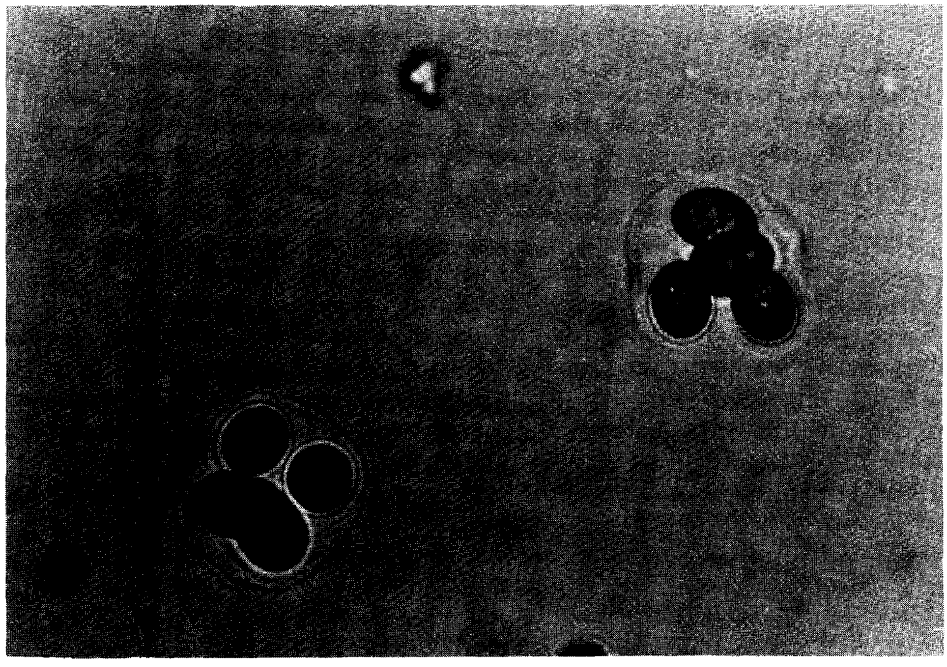
6. DePauw, N., H. Verlet & L. DeLeenheer. 1980. Heated and unheated outdoor cultures of marine algae with animal manure. In: *Algae Biomass.* Shelef, G. & C.J. Soeder (eds.). Elsevier/North-Holland Biomedical Press. pp. 315-341.

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

Oocystis pusilla

Strain: Walker Lake (S/OOCYS-1)

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



Oocystis pusilla cells (Scale: 1 cm = 10 μm)

Collection site: Walker Lake, California, USA (1)

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

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

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

Growth rate at optimum: not determined

Culture conditions:

Vitamins required: not determined

Available nitrogen sources: urea, nitrate, ammonium

Suitable media: Walker Lake

Temperature range: $15^{o}C$ - $33^{o}C$ (1)

optimum: 25-$26^{o}C$ (1)

Salinity range: 10 o/oo - 25 o/oo (1)

optimum: 18 o/oo (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:

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

References (number available in each category):

Physiology:	20
Ecology:	34
Culture:	2
Chemical composition:	3
Taxonomy:	0
Ultrastructure:	0
Food for higher organism:	2

Literature cited:

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

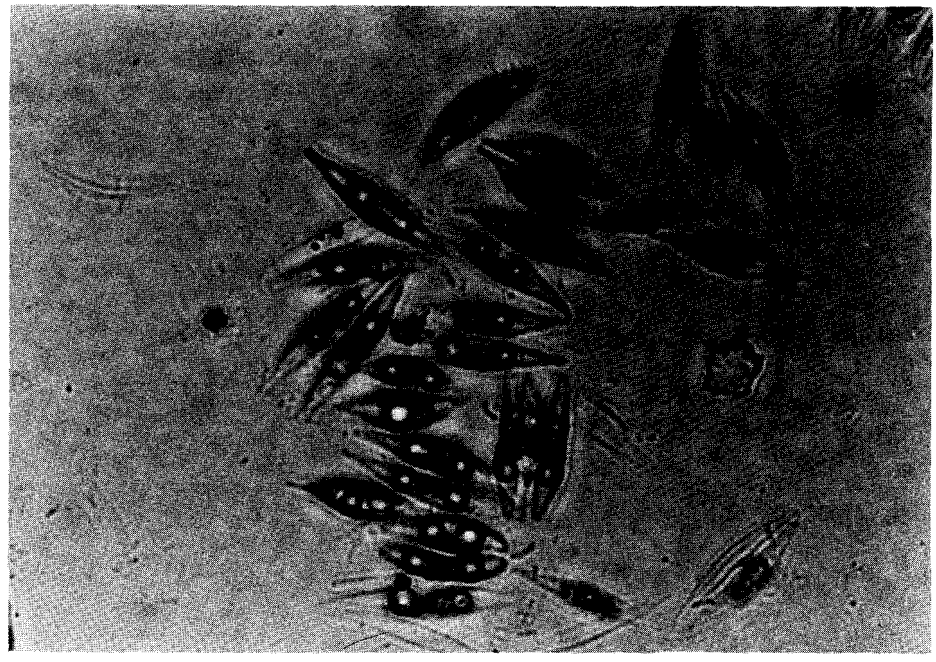
2. *SERI Aquatic Species Program 1983 Annual Report*. Solar Energy Research Institute. Publ. SERI/PR-231-2271.

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: TFX-1 (S/PHAEO-1)

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



Phaeodactylum tricornutum (TFX-1) cells containing droplets of storage lipids. (Scale: 1 cm = 10 μm)

Collection site: Woods Hole, MA, USA

Size: 15-22 μm x 3-4 μm

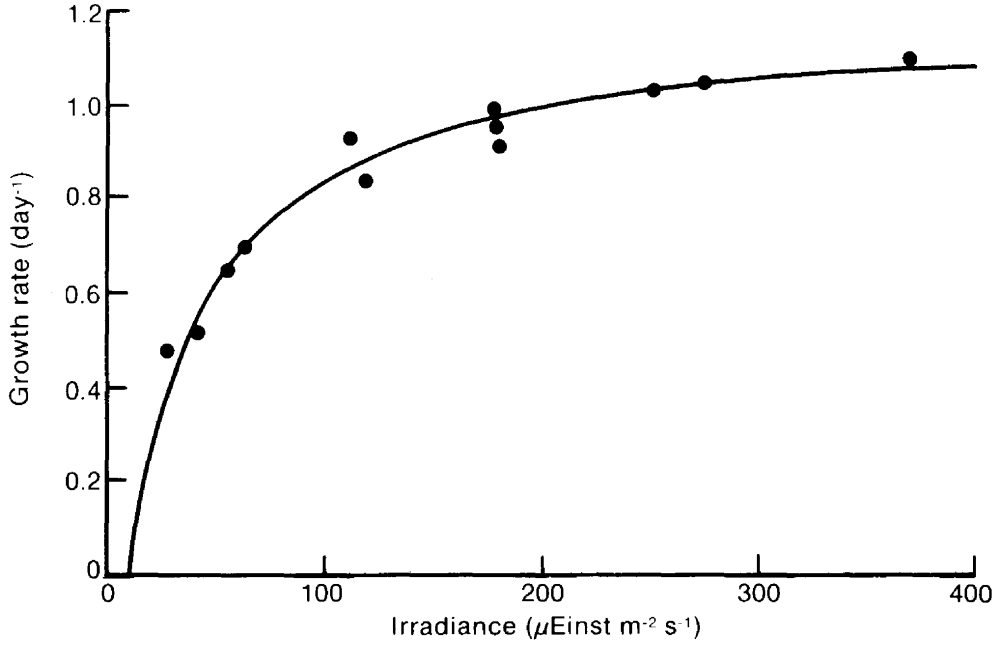
Growth form: Unicells

Growth rate at optimum (or maximum recorded): 1.36 day^{-1} (12L:12D) (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°C-27°C (1)
optimum:	24°C (1)
Salinity range:	<20 o/oo - 70 o/oo (1)
optimum:	35 o/oo (1)

Light curve of growth:



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

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

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

Fuel production options:

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

Total Energy content: 28.913 MJ/kg dry weight

Physiological notes:

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

Life cycle:

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

Outdoor culture history:

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

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

2. In the late 1970's in Belgium, outdoor cultures which were enriched with animal manure were dominated by *P. tricornutum* and *Skeletonema costatum* when culture temperatures were below 20°C. Production was 1-10 g DW m^{-2} day^{-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 which had previously occurred. (11)

B. Strain TFX-1

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

References (identical to references for Thomas strain; number available in each category):

Physiology:	104
Ecology:	86
Culture:	39
Chemical Composition:	9
Taxonomy:	0
Ultrastructure:	0
Food for Higher Organisms:	53

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.

6. Ansell, A.D., J.E.G. Raymont, K.F. Lander, E. Crowley, & P. Shackley. 1963. Studies on the mass culture of *Phaeodactylum*. II. The growth of *Phaeodactylum* and other species in outdoor tanks. *Limnol. Oceanogr.* 8:184-206.

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.

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

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.

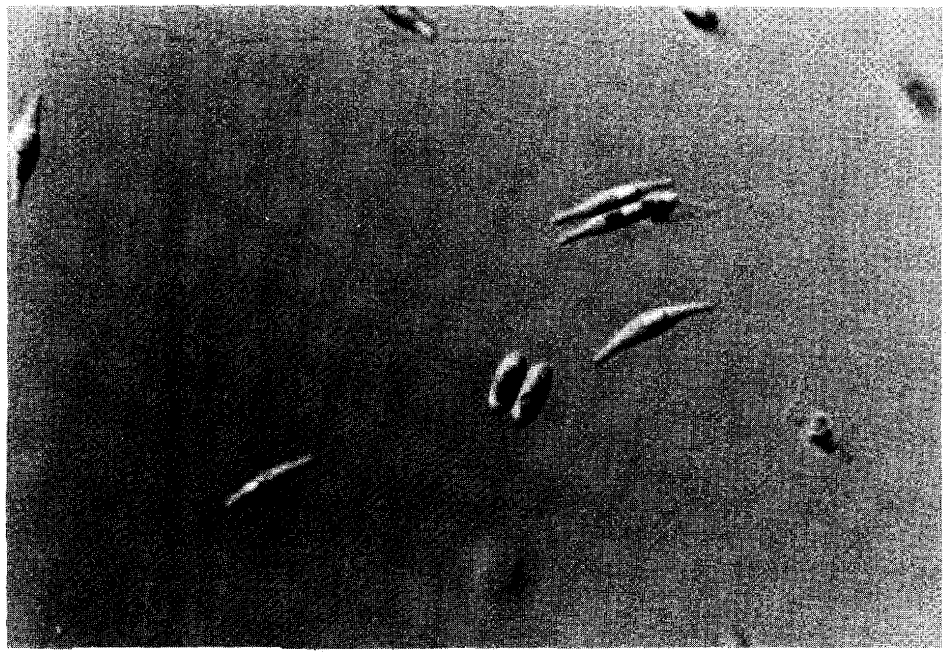
12. Mann, R. 1978. Growth of *Mytilus edulis* in a waste recycling aquaculture system. *Aquaculture*. 13:351-354.

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: Thomas (=BB) (S/PHAEO-2)

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



Fusiform and ovoid cells of *Phaeodactylum tricornutum* BB.
(Scale: 1 cm = 10 μm)

Source: W. Thomas, Scripps Institute

Size: fusiform cells = 15 μm x 4 μm

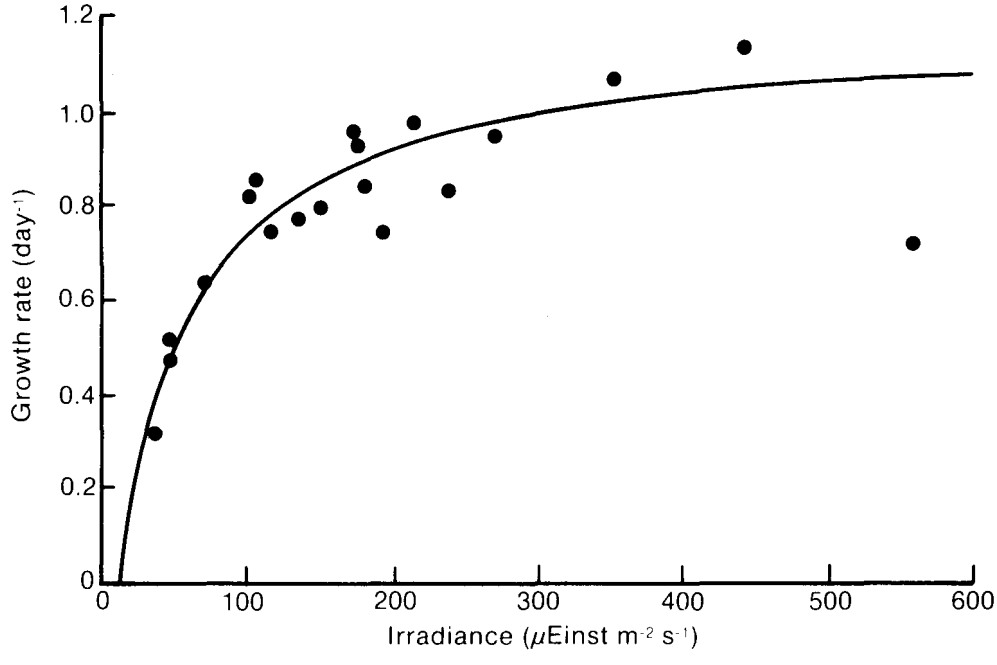
Growth form: Unicells, chains (laterally attached)

Growth rate at optimum (or maximum recorded): 1.14 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 o/oo - 70 o/oo (3)
optimum:	35 o/oo (3)

Light curve of growth:



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

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

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

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

Fuel production options:

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

Total energy content: 25.3 MJ/kg dry weight

Physiological notes:

1. Strain BB differs significantly from TFX-1 with respect to a large number of physiological parameters (8).

Life cycle:

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

Outdoor culture history: (See *P. tricornutum* TFX-1 for culture histories of other strains)

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

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

References (identical to references for TFX-1 strain; number available in each category):

Physiology:	104
Ecology:	86
Culture:	39
Chemical composition:	9
Taxonomy:	0
Ultrastructure:	0
Food for higher organisms:	53

Literature cited:

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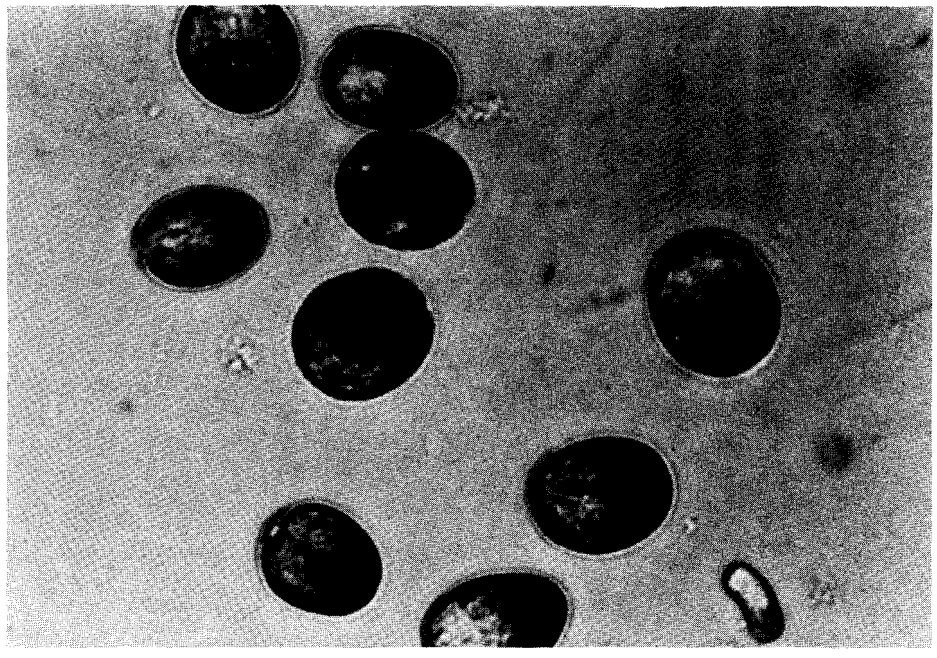
Platymonas sp.

Strain: Hawaii (S/PLATY-1)

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



Platymonas sp. cells. (Scale: 1 cm = 10 μm)

Collection site: Invaded raceway mass culture, Hawaii

Date: Summer 1983

Size: 13-18 μm x 13 μm

Growth form: unicellular

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

Culture conditions:

Vitamins required: none

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

Suitable media: not determined: grown outdoors with seawater drawn from well through coral, enriched with NH_4^+(0.5-1 mM), $PO_4 \equiv$30.05-0.1 mM), f/2 metals (1-10X recommended concentrations), and $NaHCO_3$ (equimolar to NH_4^+).

Nutritional modes: autotrophic

Temperature range: not determined

optimum: 34°C (1)

Salinity range: 15 o/oo - ? o/oo (1)

optimum: 35 o/oo (1)

Chemical composition:

Growth conditions	% lipid	% protein	% carbohydrate	Ref.	Basis
SC	18	46	36	1	AFDW
SC, N(N,P)	15	24	61	1	AFDW

Lipid composition: 33% neutral lipids (1)

Fuel production options:

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

Total energy content: 23.7 MJ/kg dry weight

Physiological note: optimum pH = 7.0 (1)

Life cycle:

Asexual reproduction by longitudinal division to form two or four daughter cells. Some species of *Platymonas* are known to form resting spores or cysts. (2)

Outdoor culture history:

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

References (number available in each category):

Physiology:	49
Ecology:	17
Culture:	19
Chemical composition:	9
Taxonomy:	4
Ultrastructure:	2
Food for higher organisms:	20

Literature cited:

1. Laws, E.A. 1984. Optimization studies in shallow algal mass culture flumes. In: *Aquatic Species Program Review: Proceedings of the April 1984 Principal Investigators Meetings.* Solar Energy Research Institute, Publ. SERI/CP-231-2341. pp. 108-132.

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

Appendix

CULTURE MEDIA

ASW Medium
(Darley and Volcani, 1969)

To one liter of distilled water add:

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

Adjust to pH 8.0 before autoclaving. Autoclave separately 0.456 g KH_2PO_4 in 100 ml distilled water, and add 10 ml/l at time of innoculation.

Trace element stock (for one liter):

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

Bolds Basal Medium
(Bischoff and Bold, 1963)

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

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

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

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

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

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

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

Adjust to pH 7.0 before autoclaving.

Botryococcus Medium
(Ben-Amotz and Tornabene, 1983)

To one liter of distilled water add:

$MgSO_4$	602 mg
$CaCl_2$	33 mg
KCl	373 mg
$NaHCO_3$	4201 mg
Na_2SiO_3 $9H_2O$	28 mg
H_3BO_3	6 mg
$FeCl_3$	0.4 mg
Na_2EDTA	11 mg
Tris	2420 mg
KNO_3	505 mg
KH_2PO_4	54 mg
Vitamin B_{12}	1.0 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 (p. 53).

f/2 Seawater
(Guillard and Ryther, 1962)

To one liter of filtered seawater add:

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

Trace elements stock solution (for 1 liter):

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

Rila Marine Mix (Rila Products, Teaneck, New Jersey) can be substituted for the seawater. Dissolve 40 g of Rila Marine Mix in one liter of distilled water.

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

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

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

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

PII trace element stock (for 1 liter):

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

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

Soil Extract:

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

Rila Marine Mix (Rila Products, Teaneck, New Jersey) can be substituted for the seawater. Dissolve 30 g Rila Marine Mix in 750 ml of distilled water.

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

To one liter of distilled water add:

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

Adjust to pH 7.0 with KOH.

For f/2 trace elements stock solution, see f/2 seawater medium (p. 53).

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

To one liter of distilled water add:

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

Final pH should be adjusted to 9.3-9.7.

Trace elements stock (for 1 liter):

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

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

To one liter of distilled water add:

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

Final pH should be adjusted to 9.3-9.7.

For Mono Lake trace elements solution, see Mono Lake medium (p. 56).

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

To one liter of distilled water add:

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

For Mono Lake trace elements solution, see Mono Lake medium (p. 56).

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