Microalgae Culture Collection
1984-1985

September 1984

Prepared by the
Microalgal Technology Research Group

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A Division of Midwest Research Institute
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Prepared for the
U.S. Department of Energy
Contract No. DE-AC02-83CH10093
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# Table of Contents

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Introduction</td>
<td>1</td>
</tr>
<tr>
<td>Explanatory Notes</td>
<td>2</td>
</tr>
<tr>
<td>Requests for Cultures</td>
<td>4</td>
</tr>
<tr>
<td><strong>List of Strains</strong></td>
<td></td>
</tr>
<tr>
<td><em>Ankistrodesmus falcatus</em></td>
<td>5</td>
</tr>
<tr>
<td><em>Botryococcus braunii</em></td>
<td>10</td>
</tr>
<tr>
<td><em>Chaetoceros gracilis</em></td>
<td>14</td>
</tr>
<tr>
<td><em>Chlorella sp.</em></td>
<td>17</td>
</tr>
<tr>
<td><em>Isochrysis aff. galbana</em></td>
<td>21</td>
</tr>
<tr>
<td><em>Nannochloropsis salina</em></td>
<td>25</td>
</tr>
<tr>
<td><em>Nitzschia sp.</em></td>
<td>29</td>
</tr>
<tr>
<td><em>Oocystis pusilla</em></td>
<td>33</td>
</tr>
<tr>
<td><em>Phaeodactylum tricornutum TFX-1</em></td>
<td>36</td>
</tr>
<tr>
<td><em>Phaeodactylum tricornutum BB</em></td>
<td>41</td>
</tr>
<tr>
<td><em>Platymonas sp.</em></td>
<td>46</td>
</tr>
<tr>
<td><strong>Appendix - Culture Media</strong></td>
<td></td>
</tr>
</tbody>
</table>

iii
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 (CO₂) limitation

L(n) light limited, where n is the culture irradiance in μEinsteins 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
Microalgal 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: 5.0‰
- pH: 9.1

Size: 35-57 μm x 3 μm

Growth form: unicells

Growth rate at optimum (or maximum recorded): 1.06 day⁻¹ (20°C)* (2)

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

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°C - 31°C (1)
    optimum: 26°C (1)
Salinity range: 1 o/oo - 10 o/oo (1)
    optimum: 7 o/oo (1)

Chemical composition:

<table>
<thead>
<tr>
<th>Growth conditions</th>
<th>% lipid</th>
<th>% protein</th>
<th>% carbohydrate</th>
<th>Ref.</th>
<th>Basis</th>
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<tr>
<td>B</td>
<td>27.2</td>
<td>34.0</td>
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<td>5</td>
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Lipid composition: (6)

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<th>Benzene</th>
<th>Chloroform</th>
<th>Acetone</th>
<th>Methanol</th>
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</thead>
<tbody>
<tr>
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<td>---</td>
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<td>9.8</td>
<td>72.6</td>
<td>19.5</td>
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<tr>
<td>B, N(N)</td>
<td>---</td>
<td>3.3</td>
<td>13.5</td>
<td>66.5</td>
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<tr>
<td>B, N(C)</td>
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<td>5.8</td>
<td>14.1</td>
<td>66.8</td>
<td>10.5</td>
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</table>
A. falcatus

Fuel production options:

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<tr>
<th>Option</th>
<th>Methane MJ/kg</th>
<th>Ester MJ/kg</th>
<th>Hydro-carbon MJ/kg</th>
<th>Ethanol MJ/kg</th>
<th>Total Recovered MJ/kg</th>
<th>Fraction Utilized</th>
<th>Liquid Fuel MJ/kg</th>
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<tbody>
<tr>
<td>1</td>
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</table>

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)
A. falcatus

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:


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
B. braunii

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:

<table>
<thead>
<tr>
<th>Growth conditions</th>
<th>% lipid</th>
<th>% protein</th>
<th>% carbohydrate</th>
<th>Ref.</th>
<th>Basis</th>
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Lipid composition: (1)

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<tr>
<th>Growth conditions</th>
<th>Hexane</th>
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<th>Chloroform</th>
<th>Acetone</th>
<th>Methanol</th>
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<td>3.4</td>
<td>21.6</td>
<td>7.4</td>
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**Fuel production options:**

<table>
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<tr>
<th>Option</th>
<th>Methane MJ/kg</th>
<th>Ester MJ/kg</th>
<th>Hydrocarbon MJ/kg</th>
<th>Ethanol MJ/kg</th>
<th>Total Recovered MJ/kg</th>
<th>Fraction Utilized</th>
<th>Liquid Fuel MJ/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>21.4</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>21.4</td>
<td>0.755</td>
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<tr>
<td>2</td>
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<td>21.4</td>
<td>0.755</td>
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<td>1.4</td>
<td>16.3</td>
<td>0.577</td>
<td>13.3</td>
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</tbody>
</table>

**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

_B. braunii_
Literature cited:


**Chaetoceros gracilis** Schutt

**Strain:** (S/CHAET-1)

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

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)
C. gracilis

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:

<table>
<thead>
<tr>
<th>Growth conditions</th>
<th>% lipid</th>
<th>% protein</th>
<th>% carbohydrate</th>
<th>Ref.</th>
<th>Basis</th>
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</thead>
<tbody>
<tr>
<td>MC</td>
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<td>48.6</td>
<td>----</td>
<td>4</td>
<td>DW</td>
</tr>
</tbody>
</table>

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):

<table>
<thead>
<tr>
<th>Category</th>
<th>Number</th>
</tr>
</thead>
<tbody>
<tr>
<td>Physiology</td>
<td>83</td>
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<tr>
<td>Ecology</td>
<td>150</td>
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<td>Culture</td>
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<td>14</td>
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<td>Taxonomy</td>
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<td>Ultrastructure</td>
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<tr>
<td>Food for higher organisms</td>
<td>49</td>
</tr>
</tbody>
</table>

Literature cited:


4. Hirata, J. Personal communication.


**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
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:

<table>
<thead>
<tr>
<th>Growth conditions</th>
<th>% lipid</th>
<th>% protein</th>
<th>% carbohydrate</th>
<th>Ref.</th>
<th>Basis</th>
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<tr>
<td>1 week old agar plate</td>
<td>13-20</td>
<td>42-51</td>
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Fuel production options:

<table>
<thead>
<tr>
<th>Option</th>
<th>Methane MJ/kg</th>
<th>Ester MJ/kg</th>
<th>Hydrocarbon MJ/kg</th>
<th>Ethanol MJ/kg</th>
<th>Total Recovered MJ/kg</th>
<th>Fraction Utilized</th>
<th>Liquid Fuel MJ/kg</th>
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<td>0</td>
<td>0</td>
<td>1.6</td>
<td>6.3</td>
<td>0.208</td>
<td>1.6</td>
</tr>
</tbody>
</table>

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/m²/day. (5)

3. Production of Chlorella in Asia exceeds 1000 kg of dried microalgae/month with average yield of 25-30 g/m²/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
Chlorella sp.

Literature cited:


**Isochrysis aff. galbana** Green

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

**Taxonomy:**
- Division: Chrysophyta
- Class: Prymnesiophyceae
- Order: Isochrysidales
- Family: Isochrysidiaceae

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)

*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.*
**I. aff. galbana** T-ISO

**Culture conditions:**

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

**Light curve of growth:**

![Light curve of growth graph]

*Data after (1)*
Chemical composition:

<table>
<thead>
<tr>
<th>Growth conditions</th>
<th>% lipid</th>
<th>% protein</th>
<th>% carbohydrate</th>
<th>Ref.</th>
<th>Basis</th>
</tr>
</thead>
<tbody>
<tr>
<td>SC?</td>
<td>7.1</td>
<td>37.0</td>
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<td></td>
<td>AFDW</td>
</tr>
<tr>
<td>SC?, N(N)</td>
<td>26.0</td>
<td>23.3</td>
<td>20.5</td>
<td></td>
<td>AFDW</td>
</tr>
<tr>
<td>SC*</td>
<td>20</td>
<td>21</td>
<td>14</td>
<td>3</td>
<td>AFDW</td>
</tr>
<tr>
<td>SC, N(N)*</td>
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<td>25</td>
<td>3</td>
<td>AFDW</td>
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</table>

Lipid composition:

<table>
<thead>
<tr>
<th>Growth Conditions</th>
<th>Hexane</th>
<th>Benzene</th>
<th>Chloroform</th>
<th>Acetone</th>
<th>Methanol</th>
<th>Ref.</th>
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</thead>
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<tr>
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<tr>
<td>SC?, N(N)</td>
<td>2.2</td>
<td>28.4</td>
<td>18.0</td>
<td>26.1</td>
<td>25.3</td>
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<td>31.6</td>
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<tr>
<td>SC, N(N)*</td>
<td>2.5</td>
<td>35.6</td>
<td>12.7</td>
<td>28.0</td>
<td>21.2</td>
<td>3</td>
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Fuel production options:

<table>
<thead>
<tr>
<th>Option</th>
<th>Methane MJ/kg</th>
<th>Ester MJ/kg</th>
<th>Hydro-carbon MJ/kg</th>
<th>Ethanol MJ/kg</th>
<th>Total Recovered MJ/kg</th>
<th>Fraction Utilized</th>
<th>Liquid Fuel MJ/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
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</tr>
</tbody>
</table>

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:


Nannochloropsis salina Hibberd

Strain: GSBSTICHO (S/NANNO-1)

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

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:

<table>
<thead>
<tr>
<th>Growth conditions</th>
<th>% lipid</th>
<th>% protein</th>
<th>% carbohydrate</th>
<th>Ref.</th>
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Lipid composition: (5)

<table>
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<tr>
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<th>Benzene</th>
<th>Chloroform</th>
<th>Acetone</th>
<th>Methanol</th>
</tr>
</thead>
<tbody>
<tr>
<td>B</td>
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<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>B, N(N)</td>
<td>2.5</td>
<td>12.4</td>
<td>25.4</td>
<td>28.7</td>
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</tr>
<tr>
<td></td>
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<td>4</td>
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</table>
**Fuel production options:**

<table>
<thead>
<tr>
<th>Option</th>
<th>Methane MJ/kg</th>
<th>Ester MJ/kg</th>
<th>Hydrocarbon MJ/kg</th>
<th>Ethanol MJ/kg</th>
<th>Total Recovered MJ/kg</th>
<th>Fraction Utilized</th>
<th>Liquid Fuel MJ/kg</th>
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</thead>
<tbody>
<tr>
<td>1</td>
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<td>8.9</td>
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<td>1.6</td>
<td>20.5</td>
<td>0.650</td>
<td>17.0</td>
</tr>
</tbody>
</table>

**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:


Nitzschia sp.

Strain: Mono Lake (S/NITZS-1)

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

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)

\[
\text{optimum: } 30-36°C \quad (1)
\]

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

\[
\text{optimum: } 50 o/oo - 70 o/oo \quad (1)
\]

Chemical composition:

<table>
<thead>
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<th>Growth conditions</th>
<th>% lipid</th>
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<th>% carbohydrate</th>
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Lipid composition: (2)

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<th>Methanol</th>
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<td>51.2</td>
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</tbody>
</table>
**Nitzschia sp.**

**Fuel production options:**

<table>
<thead>
<tr>
<th>Option</th>
<th>Methane MJ/kg</th>
<th>Ester MJ/kg</th>
<th>Hydrocarbon MJ/kg</th>
<th>Ethanol MJ/kg</th>
<th>Total Recovered MJ/kg</th>
<th>Fraction Utilized</th>
<th>Liquid Fuel MJ/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
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<td>5.9</td>
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<td>1.4</td>
<td>12.7</td>
<td>0.653</td>
<td>7.4</td>
</tr>
</tbody>
</table>

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 protozoa. (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:


Oocystis pusilla

Strain: Walker Lake (S/OOCYS-1)

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

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

Date: October 1982
Water Temperature: 18°C
Salinity: 10.6 °/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°C - 33°C (1)

optimum: 25-26°C (1)

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

optimum: 18 o/oo (1)

Chemical composition:

<table>
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<tr>
<th>Growth conditions</th>
<th>% lipid</th>
<th>% protein</th>
<th>% carbohydrate</th>
<th>Ref.</th>
<th>Basis</th>
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<tbody>
<tr>
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<td>37</td>
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</table>

Fuel production options:

<table>
<thead>
<tr>
<th>Option</th>
<th>Methane MJ/kg</th>
<th>Ester MJ/kg</th>
<th>Hydrocarbon MJ/kg</th>
<th>Ethanol MJ/kg</th>
<th>Total Recovered MJ/kg</th>
<th>Fraction Utilized</th>
<th>Liquid Fuel MJ/kg</th>
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</thead>
<tbody>
<tr>
<td>1</td>
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<td>3.6</td>
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<td>3.6</td>
<td>9.3</td>
<td>0.483</td>
<td>3.6</td>
</tr>
</tbody>
</table>

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:


Phaeodactylum tricornutum Bohlin

Strain: TFX-1 (S/PHAE0-1)

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)
**P. tricornutum TFX-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:**

![Graph showing the light curve of growth at 25°C with light of 5600K color temperature, nitrogen supplied as NH₄⁺ (1,2).](image-url)
P. tricornutum TFX-1

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

<table>
<thead>
<tr>
<th>Growth conditions</th>
<th>% lipid</th>
<th>% protein</th>
<th>% carbohydrate</th>
<th>Ref.</th>
<th>Basis</th>
</tr>
</thead>
<tbody>
<tr>
<td>SC</td>
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<td>34.5</td>
<td>15.3</td>
<td>1,2</td>
<td>C</td>
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<tr>
<td>SC, L(28)</td>
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<td>1,2</td>
<td>C</td>
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<tr>
<td>C(.271), N(P), 21.5°C</td>
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<td>27.1</td>
<td>20.4</td>
<td>1</td>
<td>C</td>
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<td>15.0</td>
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<td>C</td>
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Fuel production options:

<table>
<thead>
<tr>
<th>Option</th>
<th>Methane MJ/kg</th>
<th>Ester MJ/kg</th>
<th>Hydrocarbon MJ/kg</th>
<th>Ethanol MJ/kg</th>
<th>Total Recovered MJ/kg</th>
<th>Fraction Utilized</th>
<th>Liquid Fuel MJ/kg</th>
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<td>0</td>
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<td>0.757</td>
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<td>2</td>
<td>21.9</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>21.9</td>
<td>0.757</td>
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<tr>
<td>3</td>
<td>4.3</td>
<td>17.2</td>
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<td>22.9</td>
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<td>5</td>
<td>3.0</td>
<td>17.2</td>
<td>5.7</td>
<td>1.1</td>
<td>27.1</td>
<td>0.937</td>
<td>24.0</td>
</tr>
</tbody>
</table>

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\(^\circ\)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\(^\circ\)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:


Phaeodactylum tricornutum Bohlin

Strain: Thomas (=BB) (S/PHAE0-2)

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 NH₄⁺ (3,8).
P. tricornutum BB

Photoinhibition: 10% or more above \( \sim 500 \ \mu\text{Einsteins m}^{-2} \text{s}^{-1} \)

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:

<table>
<thead>
<tr>
<th>Growth conditions</th>
<th>% lipid</th>
<th>% protein</th>
<th>% carbohydrate</th>
<th>Ref.</th>
<th>Basis</th>
</tr>
</thead>
<tbody>
<tr>
<td>MC</td>
<td>24.6</td>
<td>---</td>
<td>---</td>
<td>4</td>
<td>C</td>
</tr>
<tr>
<td>C(.25), L</td>
<td>19.7</td>
<td>58.3</td>
<td>---</td>
<td>1</td>
<td>DW</td>
</tr>
<tr>
<td>C(.25), N(N)</td>
<td>23.2</td>
<td>19.7</td>
<td>---</td>
<td>1</td>
<td>DW</td>
</tr>
<tr>
<td>SC, L(.48)</td>
<td>34.2</td>
<td>45.3</td>
<td>9.5</td>
<td>3,8</td>
<td>C</td>
</tr>
<tr>
<td>SC</td>
<td>40.9</td>
<td>31.5</td>
<td>14.3</td>
<td>3,8</td>
<td>C</td>
</tr>
</tbody>
</table>

Fuel production options:

<table>
<thead>
<tr>
<th>Option</th>
<th>Methane MJ/kg</th>
<th>Ester MJ/kg</th>
<th>Hydrocarbon MJ/kg</th>
<th>Ethanol MJ/kg</th>
<th>Total Recovered MJ/kg</th>
<th>Fraction Utilized</th>
<th>Liquid Fuel MJ/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>18.3</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>18.3</td>
<td>0.722</td>
<td>0</td>
</tr>
<tr>
<td>2</td>
<td>18.3</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>18.3</td>
<td>0.722</td>
<td>0</td>
</tr>
<tr>
<td>3</td>
<td>16.8</td>
<td>11.1</td>
<td>3.7</td>
<td>0</td>
<td>21.7</td>
<td>0.858</td>
<td>14.8</td>
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<tr>
<td>4</td>
<td>16.8</td>
<td>11.1</td>
<td>3.7</td>
<td>1.3</td>
<td>18.1</td>
<td>0.715</td>
<td>1.3</td>
</tr>
<tr>
<td>5</td>
<td>5.4</td>
<td>11.1</td>
<td>3.7</td>
<td>1.3</td>
<td>21.5</td>
<td>0.852</td>
<td>16.1</td>
</tr>
</tbody>
</table>

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 m²) 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:


Platymonas sp.

Strain: Hawaii (S/PLATY-1)

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

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
Platymonas sp.

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 \( \text{NH}_4^+ (0.5-1 \text{ mM}), \text{PO}_4^{3-} = 30.05-0.1 \text{ mM}), f/2 \text{ metals (1-10X recommended concentrations), and NaHCO}_3 \text{ (equimolar to NH}_4^+. \text{)} \)

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:

<table>
<thead>
<tr>
<th>Growth conditions</th>
<th>% lipid</th>
<th>% protein</th>
<th>% carbohydrate</th>
<th>Ref.</th>
<th>Basis</th>
</tr>
</thead>
<tbody>
<tr>
<td>SC</td>
<td>18</td>
<td>46</td>
<td>36</td>
<td>1</td>
<td>AFDW</td>
</tr>
<tr>
<td>SC, N(N,P)</td>
<td>15</td>
<td>24</td>
<td>61</td>
<td>1</td>
<td>AFDW</td>
</tr>
</tbody>
</table>

Lipid composition: 33% neutral lipids (1)

Fuel production options:

<table>
<thead>
<tr>
<th>Option</th>
<th>Methane MJ/kg</th>
<th>Ester MJ/kg</th>
<th>Hydrocarbon MJ/kg</th>
<th>Ethanol MJ/kg</th>
<th>Total Recovered MJ/kg</th>
<th>Fraction Utilized</th>
<th>Liquid Fuel MJ/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>16.2</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>16.2</td>
<td>0.685</td>
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<td>0</td>
<td>3.5</td>
<td>15.8</td>
<td>0.667</td>
<td>3.5</td>
</tr>
<tr>
<td>5</td>
<td>6.7</td>
<td>4.7</td>
<td>2.7</td>
<td>3.5</td>
<td>17.6</td>
<td>0.743</td>
<td>10.9</td>
</tr>
</tbody>
</table>

Total energy content: 23.7 MJ/kg dry weight
Platymonas sp.

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/m²/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:


Appendix

CULTURE MEDIA
ASW Medium
(Darley and Volcani, 1969)

To one liter of distilled water add:

\[
\begin{align*}
\text{NaCl} & \quad 23.6 \text{ g} \\
\text{MgSO}_4 \cdot 7\text{H}_2\text{O} & \quad 4.9 \text{ g} \\
\text{MgCl}_2 \cdot 6\text{H}_2\text{O} & \quad 4.1 \text{ g} \\
\text{CaCl}_2 & \quad 1.1 \text{ g} \\
\text{KCl} & \quad 75 \text{ mg} \\
\text{KNO}_3 & \quad 303 \text{ mg} \\
\text{Na}_2\text{EDTA} & \quad 12 \text{ mg} \\
\text{Na}_2\text{SiO}_3 \cdot 9\text{H}_2\text{O} & \quad 40 \text{ mg} \\
\text{glycylglycine} & \quad 660 \text{ mg} \\
\text{thiamine - HCl} & \quad 0.5 \text{ mg} \\
\text{trace elements} & \quad 1.0 \text{ ml}
\end{align*}
\]

Adjust to pH 8.0 before autoclaving. Autoclave separately 0.456 g \(\text{KH}_2\text{PO}_4\) in 100 ml distilled water, and add 10 ml/l at time of inoculation.

Trace element stock (for one liter):

\[
\begin{align*}
\text{H}_3\text{BO}_3 & \quad 0.568 \text{ g} \\
\text{ZnCl}_2 & \quad 0.624 \text{ g} \\
\text{CuCl}_2 \cdot 2\text{H}_2\text{O} & \quad 0.268 \text{ g} \\
\text{Na}_2\text{MoO}_4 \cdot 2\text{H}_2\text{O} & \quad 0.252 \text{ g} \\
\text{CoCl}_2 \cdot 6\text{H}_2\text{O} & \quad 0.42 \text{ g} \\
\text{FeSO}_4 & \quad 1.36 \text{ g} \\
\text{MnCl}_2 \cdot 4\text{H}_2\text{O} & \quad 0.36 \text{ g} \\
\text{Na-tartrate} & \quad 1.77 \text{ g}
\end{align*}
\]
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:

<table>
<thead>
<tr>
<th>Salt</th>
<th>Grams</th>
</tr>
</thead>
<tbody>
<tr>
<td>NaNO₃</td>
<td>10.0</td>
</tr>
<tr>
<td>CaCl₂·2H₂O</td>
<td>1.0</td>
</tr>
<tr>
<td>MgSO₄·7H₂O</td>
<td>3.0</td>
</tr>
<tr>
<td>K₂HPO₄</td>
<td>3.0</td>
</tr>
<tr>
<td>KH₂PO₄</td>
<td>7.0</td>
</tr>
<tr>
<td>NaCl</td>
<td>1.0</td>
</tr>
</tbody>
</table>

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 H₂O (or 50 g Na₂EDTA) dissolved in 1 liter distilled H₂O).

2. 4.98 g FeSO₄·7H₂O dissolved in 1 liter of acidified water (acidified H₂O: 1.0 ml H₂SO₄ dissolved in 1 liter distilled H₂O).

3. 11.42 g H₃BO₃ dissolved in 1 liter distilled H₂O.

4. The following, in amounts indicated, all dissolved in 1 liter 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.
To one liter of distilled water add:

MgSO_{4} .............................. 602 mg
CaCl_{2} .................................. 33 mg
KCl ........................................ 373 mg
NaHCO_{3} .............................. 4201 mg
Na_{2}SiO_{3} \cdot 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 \mu g
Thiamine-HCl ............................ 0.2 \mu g
Biotin ..................................... 1.0 \mu g
f/2 trace elements stock .......... 1.0 ml

Adjust to pH 8.0

For f/2 trace elements stock solution, see f/2 seawater medium (p. 53).
f/2 Seawater
(Guillard and Ryther, 1962)

To one liter of filtered seawater add:

- $\text{NaNO}_3$ \hspace{1cm} 75 mg
- $\text{NaH}_2\text{PO}_4 \cdot \text{H}_2\text{O}$ \hspace{1cm} 5 mg
- $\text{Na}_2\text{SiO}_3 \cdot 9\text{H}_2\text{O}$ \hspace{1cm} 30 mg
- Thiamine-HCl \hspace{1cm} 100 $\mu$g
- Biotin \hspace{1cm} 0.5 $\mu$g
- $\text{B}_12$ \hspace{1cm} 0.5 $\mu$g
- Trace elements stock solution \hspace{1cm} 1 ml

Trace elements stock solution (for 1 liter):

- $\text{Na}_2\text{EDTA}$ \hspace{1cm} 4.36 g
- $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$ \hspace{1cm} 3.15 g
- $\text{MnCl}_2 \cdot 4\text{H}_2\text{O}$ \hspace{1cm} 180 mg
- $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ \hspace{1cm} 10 mg
- $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$ \hspace{1cm} 22 mg
- $\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$ \hspace{1cm} 10 mg
- $\text{NaMoO}_4 \cdot 2\text{H}_2\text{O}$ \hspace{1cm} 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.
To 750 ml of filtered seawater (28-32 o/oo salinity) add the following:

- distilled water .................. 225 ml
- \( \text{KNO}_3 \) (1M) .................. 2 ml
- \( \text{K}_2\text{HPO}_4 \) (1M) .............. 0.2 ml
- Soil extract ....................... 5 ml
- PII trace metals ................... 5 ml
- \( \text{B}_{12} \) (1 \( \mu \text{g/ml} \)) .......... 1 ml
- Thiamine-HCl (1 mg/ml) .......... 1 ml
- Biotin (2 \( \mu \text{g/ml} \)) .......... 1 ml

Autoclave the \( \text{K}_2\text{HPO}_4 \) addition separately in 10 ml of distilled water and add after the medium cools.

PII trace element stock (for 1 liter):

- \( \text{Na}_2\text{EDTA} \) .................. 6.0 g
- \( \text{FeCl}_3 \cdot 6\text{H}_2\text{O} \) .............. 0.29 g
- \( \text{H}_3\text{BO}_3 \) ...................... 6.84 g
- \( \text{MnCl}_2 \cdot 4\text{H}_2\text{O} \) .............. 0.86 g
- \( \text{ZnCl}_2 \) ....................... 0.06 g
- \( \text{CoCl}_2 \cdot 6\text{H}_2\text{O} \) .............. 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 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:

\[
\begin{align*}
\text{KNO}_3 & \quad 200 \text{ mg} \\
\text{K}_2\text{HPO}_4 & \quad 20 \text{ mg} \\
\text{MgSO}_4 \cdot \text{H}_2\text{O} & \quad 100 \text{ mg} \\
\text{CaCl}_2 \cdot 6\text{H}_2\text{O} & \quad 80 \text{ mg} \\
\text{Fe citrate} & \quad 20 \text{ mg} \\
\text{citric acid} & \quad 100 \text{ mg} \\
f/2 \text{ trace elements stock} & \quad 1 \text{ ml}
\end{align*}
\]

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:

<table>
<thead>
<tr>
<th>Compound</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>NaCl</td>
<td>26.30 g</td>
</tr>
<tr>
<td>Na₂CO₃</td>
<td>25.44 g</td>
</tr>
<tr>
<td>NaHCO₃</td>
<td>15.12 g</td>
</tr>
<tr>
<td>Na₂SO₄</td>
<td>14.20 g</td>
</tr>
<tr>
<td>KCl</td>
<td>2.91 g</td>
</tr>
<tr>
<td>H₃BO₃</td>
<td>1.92 g</td>
</tr>
<tr>
<td>KNO₃</td>
<td>1.01 g</td>
</tr>
<tr>
<td>MgSO₄</td>
<td>35 mg</td>
</tr>
<tr>
<td>Na₂SiO₃</td>
<td>198 mg</td>
</tr>
<tr>
<td>Ca(NO₃)₂</td>
<td>70 mg</td>
</tr>
<tr>
<td>KH₂PO₄</td>
<td>136 mg</td>
</tr>
<tr>
<td>Mono Lake trace elements stock</td>
<td>1 ml</td>
</tr>
<tr>
<td>1% Ferric Sequestrene</td>
<td>1 ml</td>
</tr>
</tbody>
</table>

Final pH should be adjusted to 9.3-9.7.

Trace elements stock (for 1 liter):

<table>
<thead>
<tr>
<th>Compound</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>ZnSO₄ · 7H₂O</td>
<td>84 mg</td>
</tr>
<tr>
<td>H₃BO₃</td>
<td>600 mg</td>
</tr>
<tr>
<td>CoCl₂ · 6H₂O</td>
<td>150 mg</td>
</tr>
<tr>
<td>CuSO₄</td>
<td>37 mg</td>
</tr>
<tr>
<td>MnCl₂ · 4H₂O</td>
<td>400 mg</td>
</tr>
<tr>
<td>(NH₄)₆Mo₇O₂₄ · 4H₂O</td>
<td>370 mg</td>
</tr>
</tbody>
</table>
Pyramid Lake Medium
(according to W. Thomas Scripps Institute of Oceanography)

To one liter of distilled water add:

<table>
<thead>
<tr>
<th>Compound</th>
<th>Amount (mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NaCl</td>
<td>3.271 g</td>
</tr>
<tr>
<td>NaHCO₃</td>
<td>1.176 g</td>
</tr>
<tr>
<td>MgCl₂·6H₂O</td>
<td>508 mg</td>
</tr>
<tr>
<td>Na₂CO₃</td>
<td>392 mg</td>
</tr>
<tr>
<td>CaCl₂</td>
<td>28 mg</td>
</tr>
<tr>
<td>KCl</td>
<td>246 mg</td>
</tr>
<tr>
<td>Na₂SO₄</td>
<td>207 mg</td>
</tr>
<tr>
<td>Na₂B₄O₇·10H₂O</td>
<td>9 mg</td>
</tr>
<tr>
<td>NaF</td>
<td>11 mg</td>
</tr>
<tr>
<td>NaNO₃</td>
<td>849 mg</td>
</tr>
<tr>
<td>KH₂PO₄</td>
<td>136 mg</td>
</tr>
<tr>
<td>1% Fe Sequestrene</td>
<td>1 ml</td>
</tr>
<tr>
<td>Mono Lake trace elements</td>
<td>1 ml</td>
</tr>
</tbody>
</table>

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:

<table>
<thead>
<tr>
<th>Compound</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>NaCl</td>
<td>4.075 g</td>
</tr>
<tr>
<td>NaHCO₃</td>
<td>2.184 g</td>
</tr>
<tr>
<td>Na₂CO₃</td>
<td>1.322 g</td>
</tr>
<tr>
<td>Na₂SO₄</td>
<td>3.392 g</td>
</tr>
<tr>
<td>CaCl₂</td>
<td>28 mg</td>
</tr>
<tr>
<td>MgSO₄ · 7H₂O</td>
<td>790 mg</td>
</tr>
<tr>
<td>KCl</td>
<td>430 mg</td>
</tr>
<tr>
<td>Na₂B₄O₇ · 10H₂O</td>
<td>169 mg</td>
</tr>
<tr>
<td>NaF</td>
<td>9 mg</td>
</tr>
<tr>
<td>NaNO₃</td>
<td>849 mg</td>
</tr>
<tr>
<td>KH₂PO₄</td>
<td>136 mg</td>
</tr>
<tr>
<td>1% Fe Sequestrene</td>
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</tr>
<tr>
<td>Mono Lake trace elements</td>
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</tbody>
</table>

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


