Algae Culture Workshop

Everything you ever wanted to know about algae (but were afraid to ask)

January, 2015
What is Algae?

- Phytoplankter
- Protist
- Unicellular to Macro
- Base of Marine Food Pyramid
- Multitude of species
What is Algae?

• Different colors
  – Red
  – Brown
  – Green
  – Blue/green
What is Algae?

- Different types
  - Chlorophyte
  - Flagellated
  - Diatoms
The Good, the Bad and the Ugly

- Good algae blooms
- Harmful algae blooms
- Fouling blooms
- No blooms
HAB"S on the March

- Red Tide
- Brown Tide
- Prorocentrum
- Others?
  - Ciguatera
  - Alexandrium
  - Dinophysis
  - Gymnodinium
  - Pyrodinium
  - Gonyaulax
  - Pseudo-nitzschia
Samples collected 8/24/06 by J. Bredemeyer
Analysis by R. Nuzzi

<table>
<thead>
<tr>
<th>Location</th>
<th>Cochlodinium polykrikoides</th>
<th>Gyrodinium cf. esturiale</th>
<th>Scripsiella Trochoidea</th>
<th>Peridinium Quinquecoides</th>
<th>Flagellate (3-5 um)</th>
<th>Flagellate (10 um)</th>
<th>Thalassionema nitzschoides</th>
<th>Nitzchia Sp.</th>
<th>cf. Minutocellus polymorphus</th>
</tr>
</thead>
<tbody>
<tr>
<td>SMELC Basin</td>
<td>1,820</td>
<td>110</td>
<td>30</td>
<td>20</td>
<td>1,500</td>
<td>750</td>
<td>1,000</td>
<td>7,500</td>
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<td>SMELC Dock</td>
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<td></td>
<td>70</td>
<td>1,000</td>
<td></td>
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<td>29</td>
<td>4,750</td>
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<tr>
<td>SMELC Tank</td>
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<td>10</td>
<td>40</td>
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<td>150</td>
<td>1,000</td>
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<td>2,860</td>
<td>70</td>
<td>40</td>
<td>62,222</td>
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<td>8,888</td>
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<tr>
<td>Town Creek</td>
<td>260</td>
<td>49</td>
<td>20</td>
<td>22,222</td>
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<td>8,888</td>
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</tr>
<tr>
<td>Narrow River*</td>
<td>4,800</td>
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</tr>
</tbody>
</table>

*Only Cochlodinium was enumerated.
unidentified flagellate

*Minutocellus polymorphus*

*Gyrodinium cf. estuariale*

Protoperidinium quinquecorne.

unidentified flagellate
The Macros

- Sea lettuce
- Slip gut
- Codium
- Rock weeds
- Kelp
- Filamentous species
Intensive raft culture
Open pond Spirulina farm
Palmaria palmata - Dulse
Carrageenan

- Carrageenans are a family of linear sulphated polysaccharides extracted from red seaweeds. The name is derived from a type of seaweed that is abundant along the Irish coastline. Gelatinous extracts of the *Chondrus crispus* seaweed have been used as food additives for hundreds of years. They are widely used in the food and other industries as thickening and stabilizing agents. The largest producer is the Philippines, where cultivated seaweed produces about 80% of the world supply.
Chondrus crispus – Irish moss
products using carrageenan
Porphyra yezoensis – Japanese Nori
Products using Nori
A Prefectorial Seedling Center usually has 24 tanks, giving a total facility for rearing some 200,000 shells. The tanks are not aerated but water temperature and light intensity are carefully controlled. A rise in water temperature in early summer is desirable and should not drop below 23°C too soon as this stimulates premature spore formation.
Nets of synthetic fibers are used for seeding. The most common seeding mechanism is to place the nets on drums. These are dipped into concrete tanks. About 30 nets are wound around the drums at a time and sporulating Conchocelis-phase shells are placed, still attached in strings, on the bottom of the tanks. The rotation of the drums keeps the water sufficiently turbulent to stop the conchospores from settling on the floor of the tank. One Seedling Center may seed up to 20,000 nets in one season. It takes about 10 shells adequately to seed one net.
Equipment such as boats, cutters, washers, sheet-making machine, and dryers all have a limited life of 5-10 years. Nets, ropes, frames, and rafts last 2-3 years.
Gas-driven rotary cutters are used which resemble an inverted lawnmower in construction. One or two men pull the nets over the cutter while another manages the boat. The harvesters tie and untie the nets as they pass. Nets are harvested 3-4 times.
The Nori washer consists of a cylindrical fine-mesh cage with an internal agitator. The nori plants are fed into a hopper at the top, and a powered pump with a hose feeds the washer with ample seawater.
Nori is dried into sheets. It is washed with freshwater then fed into a shredding machine, remixed with fresh water, and then fed into a machine which resembles a paper-making machine. The total Japanese output is about 7 billion sheets. Korean production is 60-100 million sheets.
Agar

• Agar is a gelatinous substance derived from seaweed. Historically and in a modern context, it is chiefly used as an ingredient in desserts throughout Japan, but in the past century has found extensive use as a solid substrate to contain culture medium for microbiological work. The gelling agent is an unbranched polysaccharide obtained from the cell membranes of some species of red algae.
• Agar was discovered by accident in Japan when some extracted seaweed was left outside the door of a mountain inn and froze overnight. Agar’s use as a solid substrate for the growth of bacteria and fungi is attributed to a laboratory assistant of Robert Koch, who saw his wife using it to make a confectionary jelly. Koch immediately saw the potential and refined the process to cultivate the tuberculosis bacillus for the first time.
Sphaerococcus euchema culture for Agar.
Workers harvesting algae for Agar
Eucheuma denticulatum being grown in farms, mainly in the Philippines
VEGETABLES from the SEA
everyday cooking with sea greens
JILL GUSMAN
Laminaria japonica - Giant kelp
The aquaculture grade micros

- T-iso
- *Tetraselmis* sp
  - Ply429
  - Chui
  - Striata
  - Plat p
- *Pavlova* sp
  - Ccmp 609
- *Chaetoceros* sp
Methods of algae culture

- Wells-Glancy method of indigenous algae
- The Milford Method for batch culture
- Continuous culture method
Aspects to algae culture

- Milford Method
  - Starts out with stock culture

Diagram:
- 250ml
- 2L Flask
- 20L Carboy
- 200L Kalwall tube
Requirements for culturing algae

- Light source
- Nutrient (F/2)
- Gas exchange (CO$_2$)
- The algae that you desire
- The equipment
- The protocol
Milford Method

Stock cultures
Inoculation to next sizes

Milford Method
Then to Carboy
Milford Method
Then to Kalwall Tube Milford Method
Aspects to algae culture

• First and foremost is cleanliness!
• Must know these things
  – Autoclave
  – Sterile transfer
  – Glassware cleaning techniques
  – Filtered water
  – Axenic culture
  – Oligoxenic culture
  – Chlorination/dechlorination techniques
  – Pasteurization
Some clean techniques

The Autoclave
Chlorination

Some clean techniques
Continuous culture

• The SeaCAPS system
  – Seasalter continuous algae culture system
  – Automated drip system for constant harvest
  – Full capability of 2000 liters/day
  – Equivalent to 10 k tubes/day
Growth phases of algae

- 1. Lag phase
- 2. Exponential growth phase
- 3. Declining Growth
- 4. Stationary phase
- 5. Death phase
Counting Algae cells

• Bright-line hemocytometer
Algae Back-ups

- Archives on agar plates or tubes
- Paste
- Milford Lab
- Other commercial sources
Summary of steps to culture algae

Step 1. Collect, filter and sterilize water, and clean culture vessel

Step 2. Add nutrients to water

Step 3. Neutralize the water (for chemical sterilization)

Step 4. Inoculate the media with appropriate algae

Step 5. Monitor algal culture for live contaminants and peak density

Step 6. Harvest algal food or use as inoculum for a new culture

Step 7. Clean, re-sterilize and inoculate culture vessels to begin again
Biofuel sources
liters/acre potential

Corn - 168 liters

Soy - 449 liters

Dandelion - 776 liters

Sunflower - 954 liters

Canola - 1,188 liters

Palm oil - 5,938 liters

Microalgae - 46,500-140,000 liters
Scientist performs laboratory tests on microalgae for biofuel
Biodiesel RV travels America
Biodiesel RV travels America

This van gets 1,300 miles per acre.
Carbon Algae Recycling System (CARS)

- FACILITY: Flue Gas and Nutrients with CO₂
- MICROALGAE POND: Water, Algae harvested, Solar Collectors, Waste Water from Municipal, Farm and Industrial sources
- BIODIGESTER: Methane back to industry
- GAS CLEANING: To Markets
- SEPARATOR / SOLIDS RECOVERY: To Markets
- BIO-FUELS: To Markets
- NUTRACEUTICALS & CHEMICALS: To Markets
- FERTILIZERS: To Markets
Typical Commercial Microalgae Production Facility, Kona, Hawaii. (This one being 90 acres).
Seawater → Carbon Dioxide → Sunlight → EXTRACION → Phycocolloids* → Biochemicals*

SEAWEEDS → FERMENTATION → Methane Alcohols Esters, Acids

Oxygen Nutrient-scubbed Effluent → PYROLYSIS → Gas Chemicals Coal-like

PYROLYSIS → Fodder* Fertiliser* Human food*