# Microalgae Production and Their Use in Animal Feeds



Gerald R. Cysewski, Ph.D. Chief Science Officer

Cyanotech Corporation

# Cyanotech

- Specializing in Microalgae Technology
- Operating since 1984
- 75 Employees
- ISO 9001:2000 Certified
- GMP Certified by NPA
- Non-GMO, environmentally friendly products
- Public Company: NASDAQ:CYAN



Keahole Point, Hawaii

# Cyanotech's Production Facility



- •90 acre facility in Kona, Hawaii.
- Consistent sun and temperature yearround
- •Separate production facilities for Spirulina and Haematococcus
- Major Spirulina production capacity
- Astaxanthin production is consistent and can be expanded to meet growing markets.
- Focus on high-value human nutrition products

# Microalgae Production In Kona, Hawaii

- Ideal Climate
  - Warm year round
  - High solar insolation
  - Low rain fall
  - Unique resource
    - Cold deep seawater
- High Costs
  - Land Clearing (hard lava)
    - •US\$ 80,000/acre
  - $-CO_2$ 
    - •US\$ 440/mt
  - Power
    - •US\$ 0.35/Kwh



# Spirulina Production

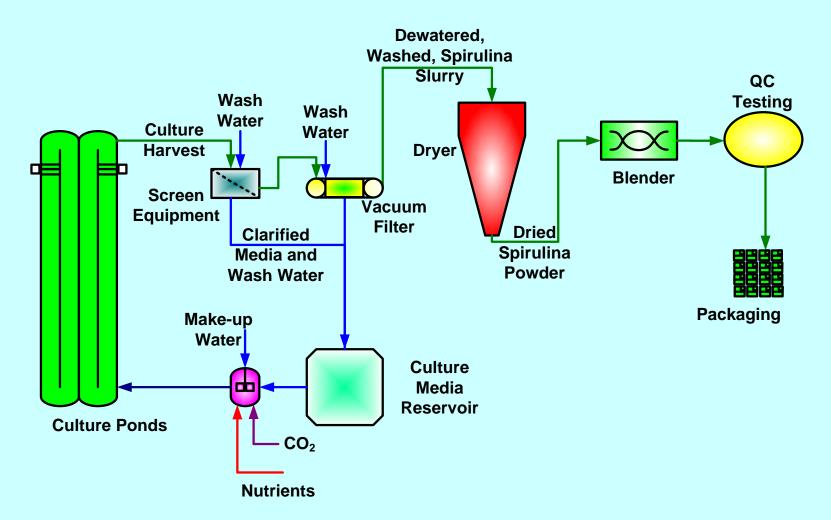


- 40 Culture ponds
- Average size2,900 sq meters
- Total area29 acres





# Spirulina Production Flow Chart





# Spirulina-High Nutritional Value

- 60% Protein
- Rich in carotenoids
  - Beta Carotene
  - Zeaxanthin
- Phycocyanin
  - Liver and Kidney protection
- Immune stimulating compounds
- Antiviral compounds



# Spirulina Feeding Studies Yellowtail—0.5% Spirulina Diet

- 20,000 fish
  - •10,000 on Spirulina diet
- •37% Increase in growth rate
- 14% Increase in survival rates
- 30% reduction in medication
- Improved coloration and quality



# Spirulina Feeding Studies Salmon—2.5% Spirulina Diet

- 23% Increase in growth rate
- Improved coloration and quality
- Improved coloration also for:
  - Sea Bream
  - Mackerel
  - •Koi
  - Other tropical aquarium fish



#### Haematococcus (Astaxanthin) Production



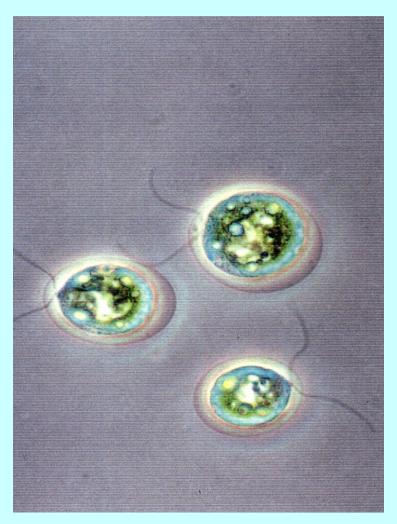
- 20 Culture ponds
- Average size2,800 sq meters
- Total area14 acres

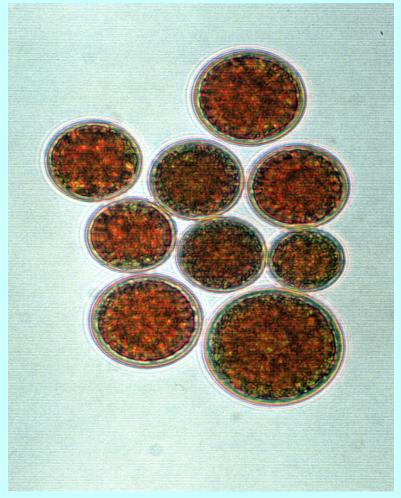


#### What is Haematococcus?

- Haematococcus is a green algae, Chlorophyta.
- •Found in pools of fresh water throughout the world.
- •Studied since 1797 (Girod-Chantrans). Astaxanthin correctly identified as red pigment in 1944 (Tisher).
- •Produces the highest concentration of natural astaxanthin (50,000 ppm. Wild type *Phaffia* produces 200 ppm astaxanthin, Krill or crawfish oils up to 1200 ppm.
- •Green cells have flagella to provide motility to seek new nutrient sources.
- •When stressed, cells lose flagella, encyst and produce intracellular astaxanthin for protection against oxygen radicals and UV light.

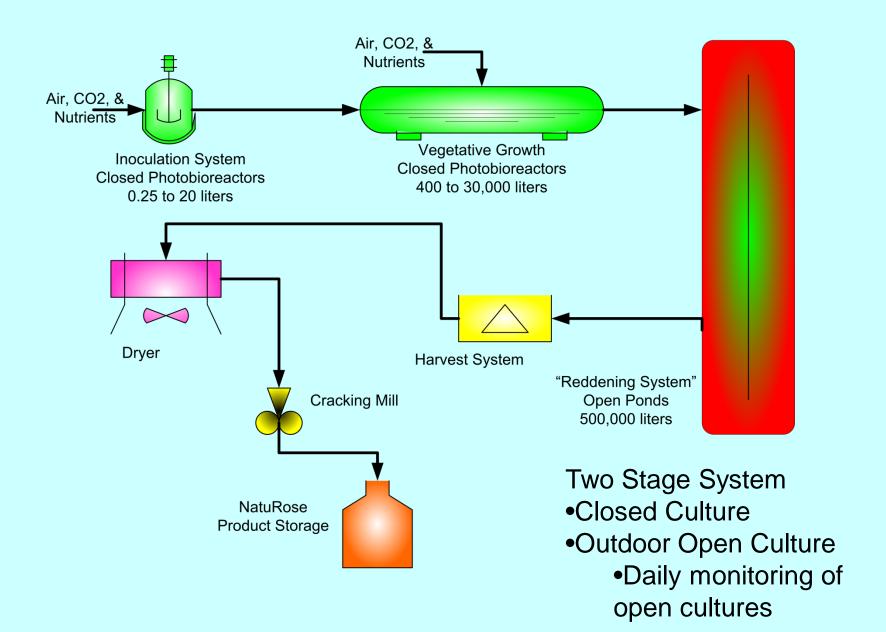
#### **Haematococcus Cells**





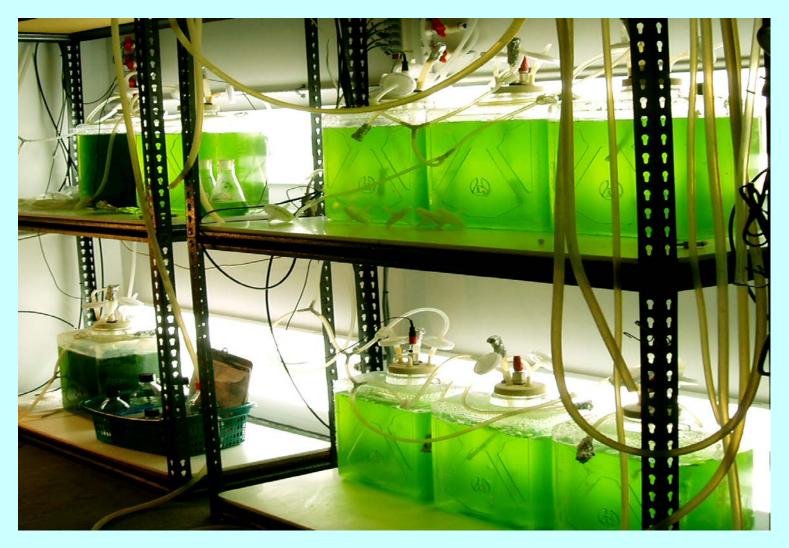
Motile, green *Haematococcus*cells

Encysted cells produce massive amounts of astaxanthin (400x magnification)





250 ml Shake Flask Cultures



20 Liter Carboy Culture System



400 Liter Column Closed Culture System

#### Astaxanthin formation in Haematococcus cultures



#### Final Production in Reddening Ponds

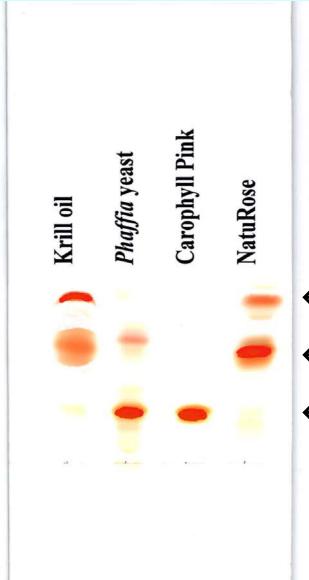


The final stage *Haematococcus* production is in 500,000 liter ponds where high concentrations of astaxanthin accumulate in cells.

# **Astaxanthin Chemistry**

- Astaxanthin is a carotenoid similar to beta-carotene, lutein and lycopene.
- Carotenoids are sensitive to heat, light and oxygen.
- Conjugated double bonds 'chromophore' elicits red color.
- •The 3-hydroxyl and 4-keto groups bind to muscle flesh of salmonid flesh. Astaxanthin is lipid soluble (lipophilic).
- •Hydroxyl groups can be esterified to fatty acids, creating mono and diesters.

### Carotenoid analysis



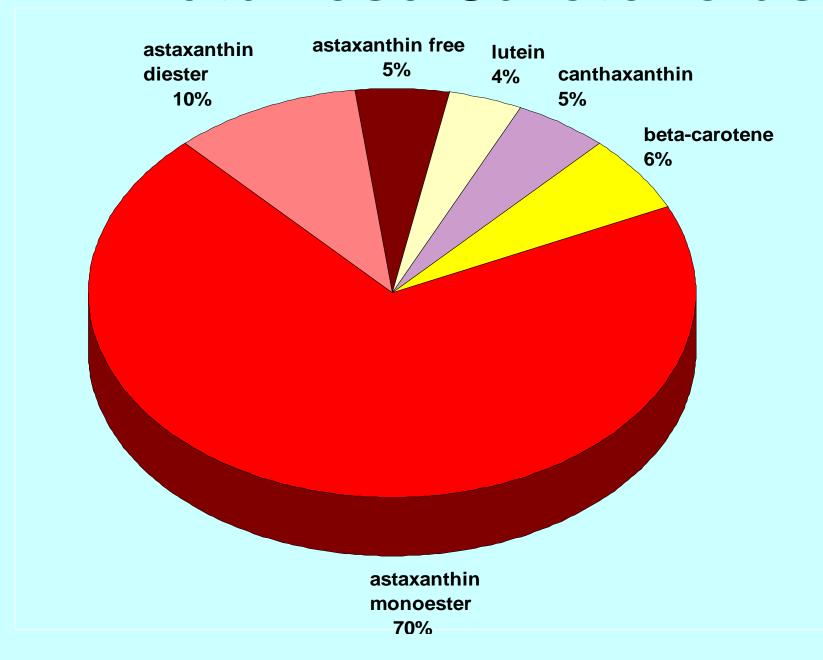
Thin-layer chromatography shows NatuRose is most similar to krill, a natural source of astaxanthin for wild salmon.

- ← Astaxanthin diesters
- ←Astaxanthin monoesters
- ← Free astaxanthin

#### What is NatuRose?

- •NatuRose<sup>™</sup> is a natural source of astaxanthin from non-GMO *Haematococcus pluvialis*. NatuRose is milled to crack cells, and spray-dried into a dark red powder.
- Consistent concentration: *Standardized* to contain 2.0% (20,000 ppm) astaxanthin, plus beta-carotene, canthaxanthin and lutein.
- Whole algae is used: lipids, proteins and carbohydrates
- Packaged in vacuum-sealed, foil laminate bags.
- •Added to feeds as a premix or can blended into oils and used to topcoat feeds.

#### **NatuRose Carotenoids**



# NatuRose Approvals



- •Approved by US FDA for salmonids (21 CFR 73.185).
- Approved by Canada CFIA for salmonids (Reg. # 990535).
- Approved in Japan for all animal feeds.
- Organic approval New Zealand.

# **NatuRose Applications**

NatuRose was used worldwide to pigment shrimp, trout, Coho, Atlantic salmon, red sea bream, tropical fish, and egg yolks.



# NatuRose Feeding Studies

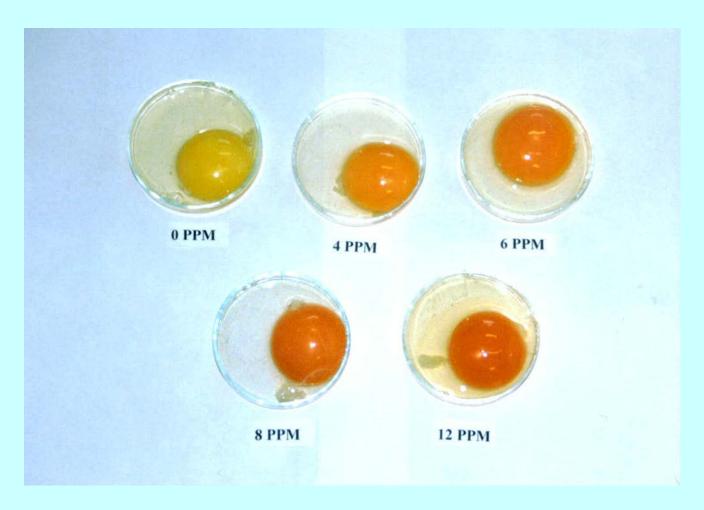
- Trout
  - •3 Studies
- Salmon
  - 4 Studies
- Shrimp
  - •6 Studies
- Yellowtail
- Sea Bream
- Ornamentals
  - •5 Studies
- Poultry
  - •3 Studies
- Dogs
  - "Secret ingredient"

## **Atlantic Salmon**





# **Poultry**



NatuRose coloration of egg yolks, University of New England

# NatuRose Safety



- 48 NatuRose Customers in 15 Different Countries
  - •Commercial aquaculture—Salmon, trout, sea bream, yellow tail
  - Ornamental fish feeds
  - Poultry
  - Dogs
- Discontinued NatuRose Sales in late 2007
  - High demand in human supplement market, BioAstin
  - •97% sales increase first 6 months of 2011
  - Expanding production by 33%

