Marine micro algae -A global perspective

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The surging usage of marine microalgae ingredients and proteins in the production of aquaculture, pharmaceutical, and nutraceutical products is set to drive sales in the market. Increasing government initiatives to standardize the cultivation process of microalgae is expected to be a key factor propelling the growth. Rapid technological advancements to develop new strains that enhance energy efficiency and handle high salinity boost the market further. The vital need for the early detection of contaminants is anticipated to create new growth opportunities for novel photobioreactor microalgae producers. *Arthrospira* and *chlorella* are the most common species of algae photobioreactors that provide high-quality proteins. These species contain amino acid compositions similar to conventional protein sources, such as soybeans and eggs. The usage of *chlorella* in hair care products is estimated to surge as it improves the keratin level and strengthens hair roots. The increasing humanization of pets is leading to the high demand for healthy foods from pet owners, thereby propelling the usage of algae photobioreactors in the pet food industry. The demand for high-quality premium pet food is expected to augment sales significantly. Algae photobioreactor contains substantial amounts of vitamins E, C, and A and Beta-carotene, making it an ideal ingredient in pet food. It helps boost the pet's appetite and strengthens its immune system, unlike regular foods available in the market. Many countries are investing on applying algae for solving wastewater treatment issues by deploying novel photobioreactor microalgae and to purify water resources and make them fit for consumption. Many prominent companies are also expected to use microalgae in the automotive industry due to pressure on fossil fuels The Microalgae Market is estimated to secure a valuation of USD 11.8 billion in 2023 and is predicted to reach USD 25.4 billion by 2033. The market is capturing a CAGR (Compound Annual Growth Rate)of 8% during the forecast period. Renowned Microalgae Companies are Introducing Spirulina Infused Products. Rising Number of Research Programs to Create Growth Opportunities for Microalgae Companies. Adoption of Novel Photobioreactor Microalgae to Grow in Wastewater Treatment Plants. Growth is attributable to this industry's surging utilization of fermented algae as a food additive.

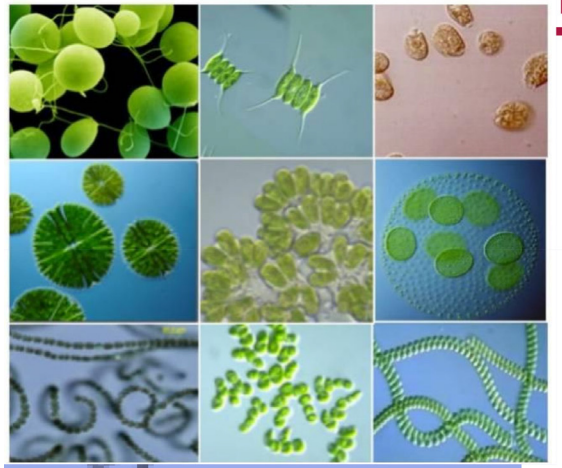
The United States	25.7%
Germany	5.3%
Japan	3.0%
Australia	2.8%
China	4.5%
India	14.8%
The United Kingdom	5%

In November 2021, Honeywell Technology declared that its UOP Ecofining technology successfully supported the world's first jet flights using sustainable aviation fuel from algal oil. Two commercial flights combined microalgae feedstock and renewable jet fuel through Ecofining. Developing other types of fuels based on fermented algae is expected to augur well for the United States market. In February 2022, Ful, an Amsterdam-based provider of sustainable and nourishing food and beverages, launched its new vivid blue-colored soda infused with spirulina. The company aims to reduce the food system's carbon footprint by using algae to make a popular ingredient. By source, the marine water segment is likely to dominate the global market in the forthcoming years. Marine microalgae can be easily produced on unproductive and desert soil using specialized growth chambers. The issue of land rivalry is also eliminated as food crops may not be fully grown in these areas. This type of microalgae does not require freshwater, which is considered an expensive and significantly diminishing resource. Microalgae can improve food products' aroma, flavor, texture, and taste. It provides high nutritional value. Thus, many food and beverage manufacturers are using it in salad dressings, snacks, meat, confectionery, soups, eggs, ice creams, sauces, and fish globally.

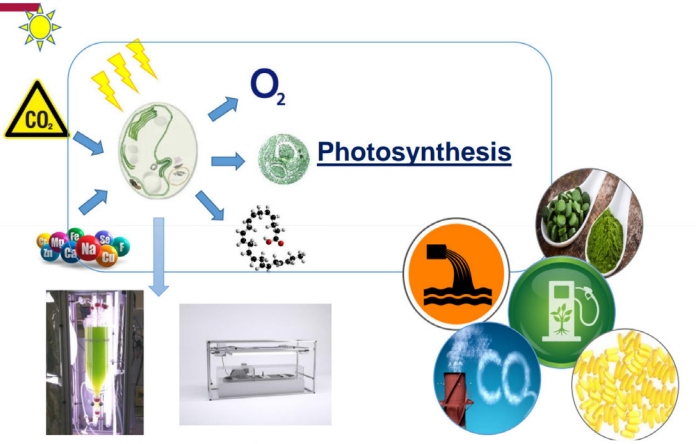
Microalgae ? Microalgae: a global view of culture systems

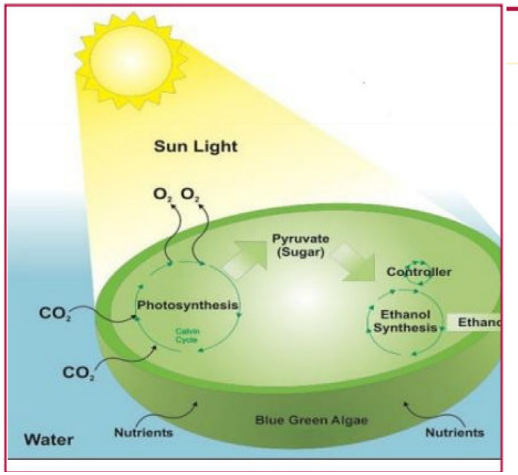
- Lower aquatic plants: photosynthetic microorganisms
- Several morphologies (0.2 to 2 mm in diameter)
- High biodiversity: 1 to 10 million algae species
- Habitat: Marine or fresh algae
- Environmental benefits (fixation of CO2, wastewater treatment)
- Pigments: chlorophyll, carotenoids and phycobiliproteins: biotechnological applications

"Phytoplankton" the first producers of oxygen essential to the majority of living beings.

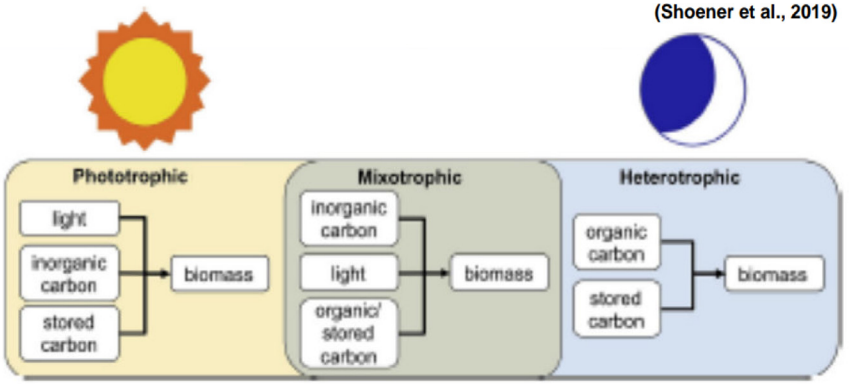


Microalgae, a photosynthetic micro-plant !

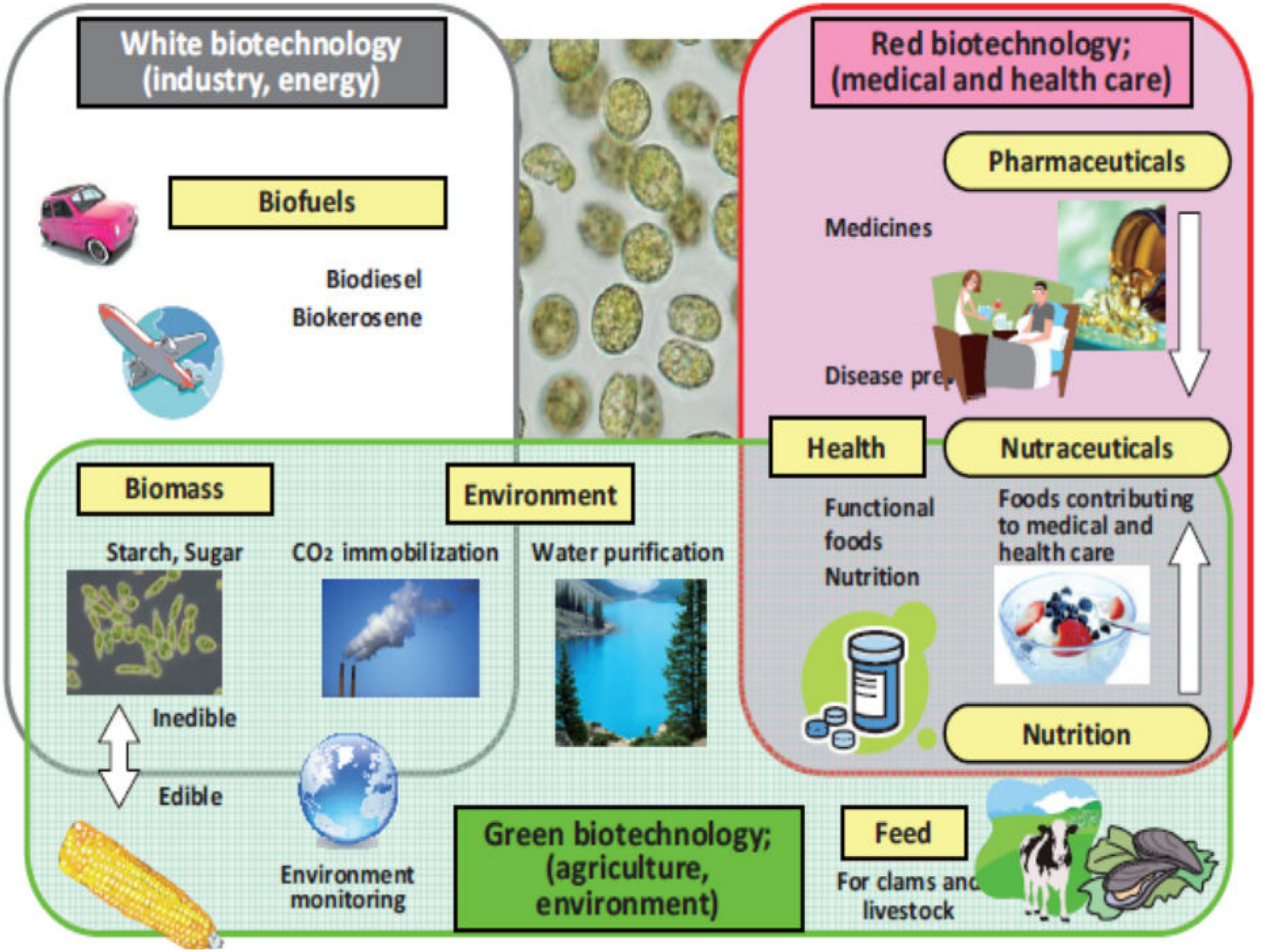




(Shoener et al., 2019)



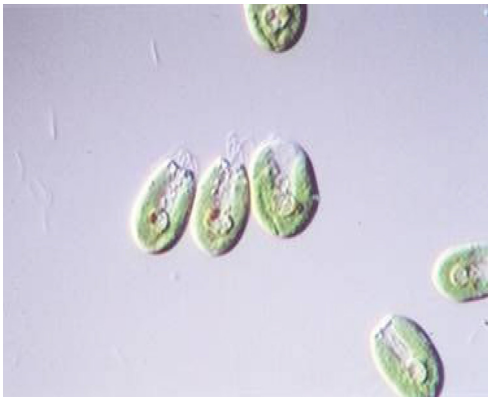
Classification based on morphology, pigmentation , chemical nature of photosynthetic product & organization of photosynthetic membranes
Diatoms (bacillariophyta), dinoflagellates (dinophyta), green and yellow-brown flagellates (chlorophyta; prasinophyta; prymnesiophyta, cryptophyta, chrysophyta and rhaphidiophyta) and blue-green algae (cyanophyta).
Micro algae are found in both benthic and littoral habitats and also throughout the ocean waters as called phytoplankton
20 µm
20 µm
20 µm
20 µm
20 µm
20 µm
50 µm
50 µm
20 µm
20 µm
50 µm
50 µm
50 µm


White biotechnology (industry, energy)
Biofuels
Biodiesel
Biokerosene
Red biotechnology; (medical and health care)
Pharmaceuticals
Medicines
Disease pre
Health
Nutraceuticals
Functional foods
Nutrition
Foods contributing to medical and health care
Nutrition
Biomass
Starch, Sugar
Inedible
Edible
Environment
CO2 immobilization
Water purification
Environment monitoring
Green biotechnology; (agriculture, environment)
Feed
For clams and livestock

Different algal species are grown for different species of fish or shrimp depending on the culture technique.

Shrimp culture	Fish culture	
Tetraselmis chuii	*Tetraselmis suesica*	*Nannochloropsis sp.*
Isochrysis galbana	*Isochrysis galbana*	*Chlamydamonas sp.*
Chaetoceros gracilis	*Monochrysis lutheri*	*Nannochloris atomus*
Skeletonema sp	*Chrorella sp.*	*Nannochloropsis oculata*
Spirulina platensis	*Spirulina platensis*	*Pavlova lutheri*
	Dunaliella sp.	*Pseudochrysis galbana*

Prasinophyceae – greenish coloured algae



Tetraselmis chuii

Large green flagellate, often used in larval rearing of shrimp, fish and shellfish

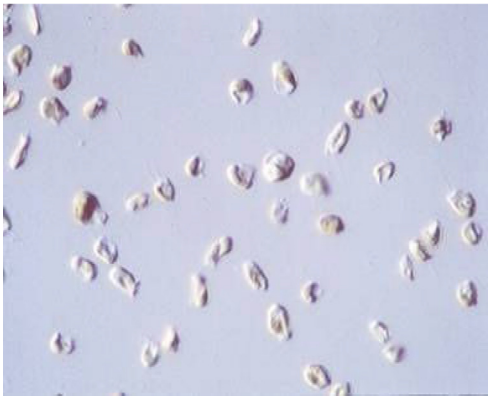
Optimal salinity 15 - 36 $^{0}/_{00}$

Optimal temperature 15 - 33°C

Typical densities in mass culture 300-450 cells/ml

Very high lipid content

Prynesiophyceae - golden brown flagellate



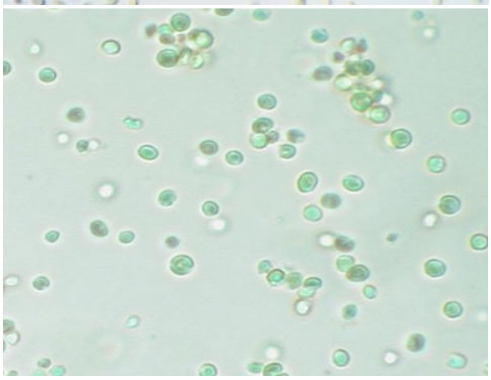
Isochrysis sp.

- Used especially as a primary algae in shellfish hatcheries
- Size: 3-5 um
- Spherical to pear shaped

Isochrysis galbana tahition

- Size: 5-6 um
- Prefer temperatures up to 30 °C and high light intensities
- High DHA level and is therefore used for growing rotifers

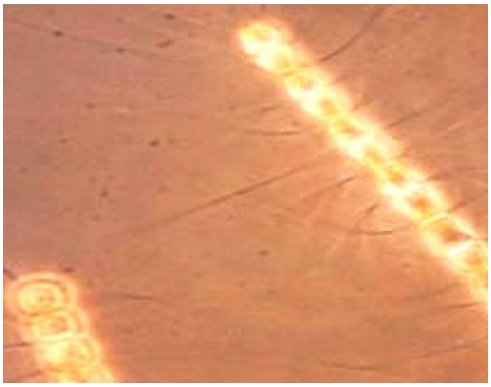
Eustigatophyceae - greenish yellow algae



Nannochloropsis

- Small green flagellate
- Size: 2-4 um
- Used in rotifer production
- Popular as greenwater
- Keeps suspended in the water column
- High total lipid content and EPA level.

Bacillariophyceae - Diatoms

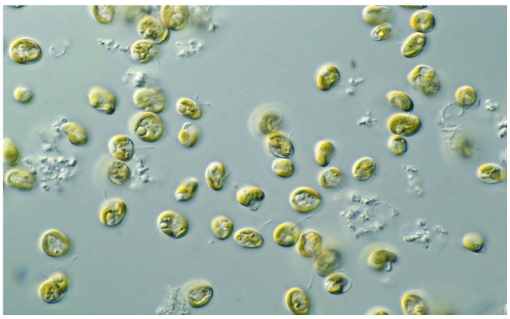


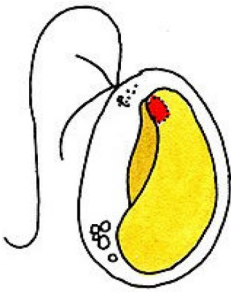
Chaetoceros sp.

- non-chain forming marine diatoms
- golden brown, rectangular in shape
- Size:4-6 um
- Optimal temperature: 25 and 30 °C
- Optimal salinity: 17 and 25 ppt
- Light intensity: 500 to 10,000 lux

Nutritional value in some species

Microalgae	Vit C (Ascorbic Acid)	Chlorophyll A	Protein	Carbohydrate	Lipid	EPA (20:5) (% of Lipid)	DHA (22:6) (% of Lipid)
Tetraselmis	0.25%	1.42%	54.66%	18.31%	14.27%	9.3%	0%
Nannochloropsis	0.85%	0.89%	52.11%	12.32%	27.64%	10.1%	0%
CHGRA	1.60%	1.04%	27.68%	23.20%	9.29%	5.0%	0.5%
T-ISO	0.4%	0.98%	46.69%	24.15%	17.07%	0.25%	8.5%





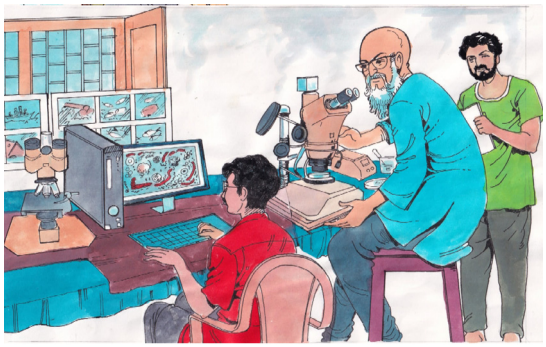
PAVLOVA

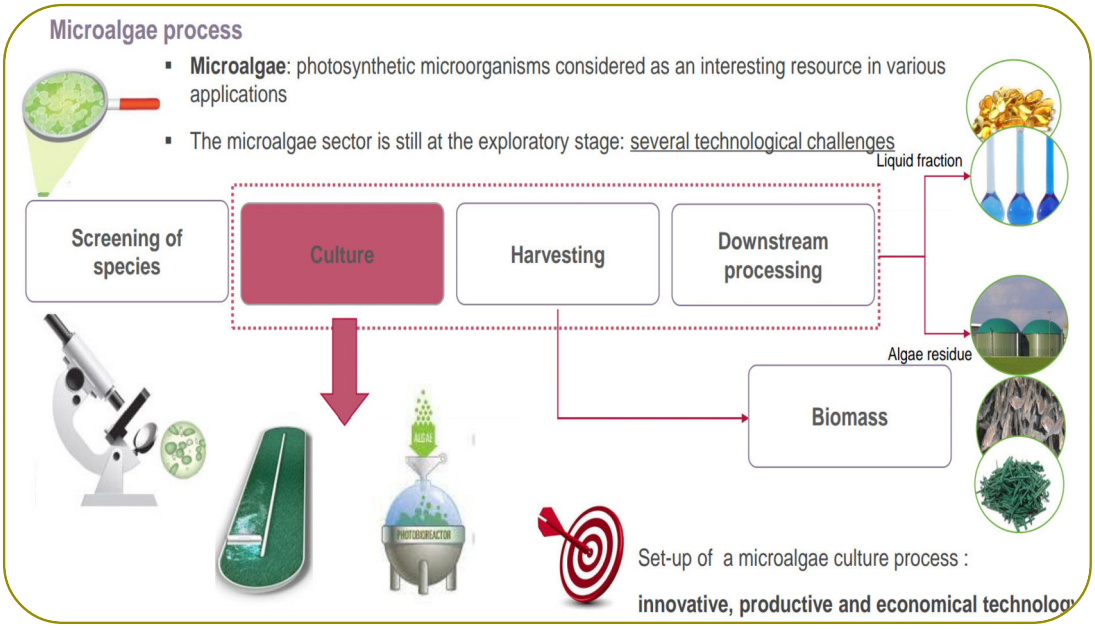
Summary algae species

Microalgae Culture	Type	Cell Size	Animals
Nannochloropsis	Green Flagellate	1.5-3 microns	Zooplankton, Marine Fish Larvae
CHGRA (Chaetoceros gracilis)	Diatom	6-9 microns	Zooplankton, Shellfish, Penaeid Shrimp
T-ISO (Isochrysis galbana)	Yellow Flagellate	5-6 microns	Zooplankton, Shellfish, Shrimp
T-ISO (Isochrysis galbana)	Yellow Flagellate	5-6 microns	Zooplankton, Shellfish, Shrimp
Pavlova (Pavlova pinguis)	Brown Flagellate	5-6 microns	Zooplankton, Shellfish
Tetraselmis	Green Flagellate	14-23 microns	Zooplankton, Shellfish, Penaeid Shrimp, Abalone Larvae

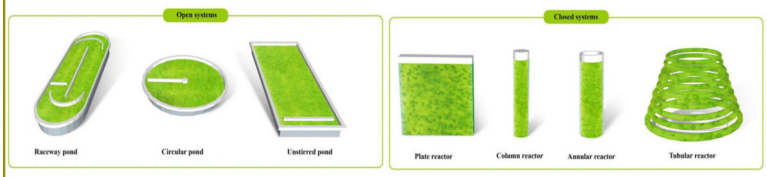
Summary algae species

Culture	Paste Concentration (cells/ml)	Typical Culture Density (cells/ml)
Tetraselmis	1.0 Billion	400,000
Nannochloropsis	51 Billion	20 Million
CHGRA	3.2 Billion	1.5 Million
T-ISO	6.2 Billion	22 Million

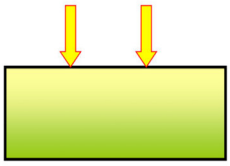




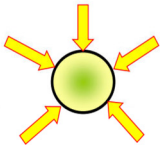
Microalgae culture systems



(Zerrouki et al., 2019)



Productivity:
0,06 – 0,1 g/L day

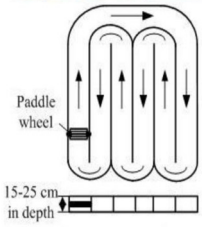


Productivity:
0,09 – 2,7 g/L day

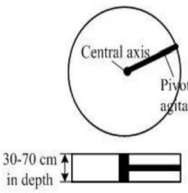
	Open system	Closed system
CO_2 biofixation yield	Low	High
Water losses	High	Low
Growth rate	Low	High
Control law	Difficult	Easy

Importance of culture system selection
Potential of hybride systems

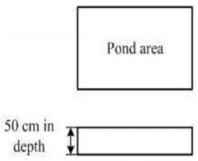
Open systems



(a) Raceway pond



(b) Circular pond

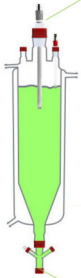


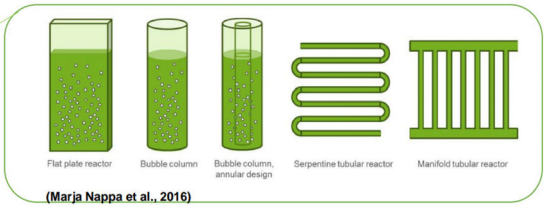
(c) Unstirred pond

(Ying Shen, 2009)

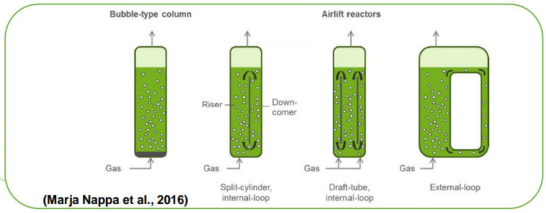
- Raceway pond: widely used for large-scale algal biomass production
- Low cost of construction and simplicity of installation and maintenance
- Biomass productivity impacted by evaporative losses, easily contaminated cultures, photoinhibition in the summer, light used by the cells, and diffusion of CO_2
- Most important design parameters of a raceway pond: the working depth and the hydraulic retention time (HRT)

Closed Systems, PBR



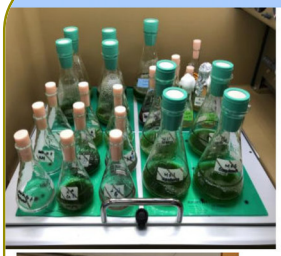


(Marja Nappa et al., 2016)



(Marja Nappa et al., 2016)

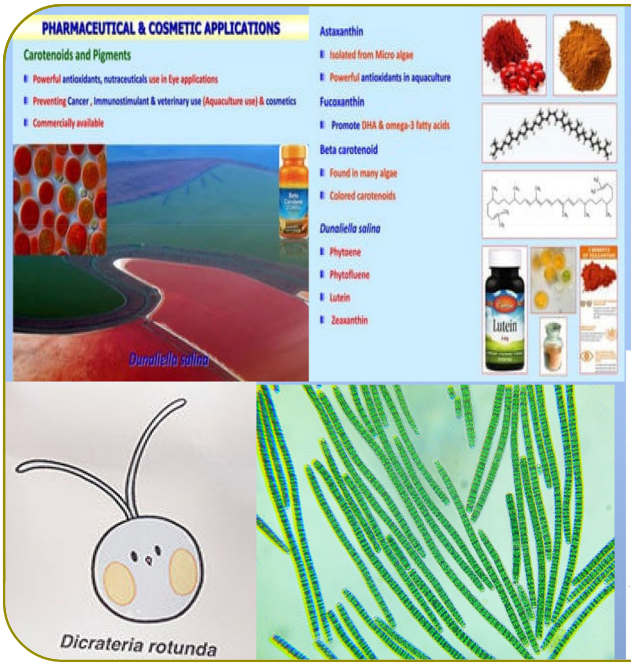
- Various design and mode of operation
- Construction materials: glass or plastic; rigid or flexible
- System adapted to algal species that cannot be grown in open systems
- Higher productivities and cost investment than ponds
- Energy requirement
- Scale-up is more difficult because of engineering issues related to gas/liquid mass transfer, energy efficient mixing and cooling of the culture



Experimental design

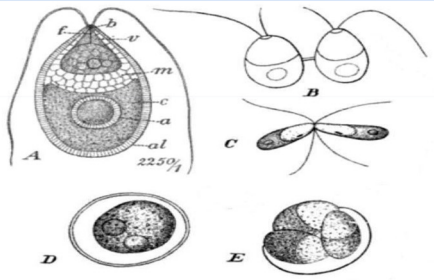
- Temperature
  - Cellular chemical composition
  - Uptake of nutriement and CO_2
  - Growth rate
- Light
  - Biomass accumulation
  - Lipid accumulation
  - Chlorophyll content

Sustainability of algae species for growing over all the year



Dunaliella β-carotene has an increasing demand and a wide variety of market applications: colouring agent in food industries; component in pharmaceutics, cosmetics and health foods; dietetic industries; diagnostics and biomedical research

D. salina is the richest algal source of β-carotene and glycerol *D. salina* can produce β-carotene up to 14% of its dry weight under conditions of high salinity, light and temperature, as well as nutrient limitation. Rich source of Phytosterols with 9 carbon atoms .



Open Recirculation pond for commercial production of marine microalgae

- Commercial scale microalgae culture are becoming more realistic for ecofriendly bioenergy production – renewable fuel.
- LC-PUFA rich vegan oil – Bio oil
- Microalgal production systems, open ponds are most widely used for large scale cultivation.

Algae paste

Mother Ponds (4 ton capacity)

Production pond (49.5 m^3)

Tubular bowl centrifuge unit

Poly house drying unit

Algae paste and meal

Positioning the paddle wheel (35degree) (15cm leaf width) – 6 leafs

Source Dr P Kalidas, PS /SIC RS of ICAR CMFRI Karwar

Nutrient or Fertilizer feeder to the culture ponds

Source Dr P Kalidas, PS /SIC RS of ICAR CMFRI Karwar

Injection of one ppm of CO_2 for 3 h per day

Source Dr P Kalidas, PS /SIC RS of ICAR CMFRI Karwar

Suction pump (2 hp) fitted with 50 micro bag filters

Source Dr P Kalidas, PS /SIC RS of ICAR CMFRI Karwar

Suction pump (2 hp) fitted with 50 micro bag filters

Source Dr P Kalidas, PS /SIC RS of ICAR CMFRI Karwar

Microalgae Harvesting unit

Bowl capacity:
6 L (SS)
16000 rpm

Filtration efficiency:
1000 L / h

Motor:
2 hp

Industrial Tubular Bowl Centrifuge

Source Dr P Kalidas, PS /SIC RS of ICAR CMFRI Karwar

Standardized Procedure for the commercial production of marine microalgae

I. Inoculums
(Stock culture to Mother culture: 2 t capacity) (4-5 days)

II. i.e
(Mass culture) (15 days to mature) - Feeding commercial fertilizer (every 5 days interval)

III. Microalgae quality and Water quality analysis (cell counts : 3 times / day)

1 ppm of CO_2 for 3 h/day. Position of paddle wheel aerator : Proper mixing and water flow

IV. Microalgae wet biomass harvesting unit
(Tubular bowl centrifuge attached to micron filters) (5 h / every alternative day) continue harvest (120 days) & recirculated of filtered seawater

V. Poly-house drying unit (Dry biomass and algae meal production) (2 days under 45-48 ^{0}C to remove 90% moisture)

VI. Package, Storage
(-2 to - 4^{0}C), (90 days)

Source Dr P Kalidas,
PS /SIC RS of ICAR CMFRI Karwar

Economics (Indian Rupees): Operational / Sensitivity Analysis

Present unit production capacity :
10 kg wet wt / day

(2.9 kg dry wt / day)

- Capital Cost (CC) = 120.00 lakhs
- Operation cost (OC): 73.8 Lakhs
- Gross Income (GI): 115.35 Lakhs
- Net income (NI) (GI-OE): 29.55 Lakhs
- Net operating income (GI-OC): 41.55 Lakhs
- Operation ratio =OC/GI = 0.64
- Net-benefit earnings ratio = NP/GI =36.33%
- Rate of return on Investment (ROI) = 33.77%
- BCR for 10% discount rate : 1.56 BCR 30%: 1.51
- IRR % : 69.25%

The given BCR is for the Open pond for commercial production of marine microalgae *Nannochloropsis gaditana,*

it may vary with the selected marine microalgae production and market price ($).

Source Dr P Kalidas,
PS /SIC RS of ICAR CMFRI Karwar

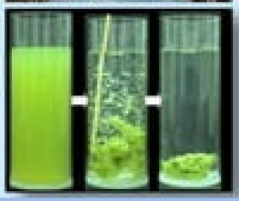
How to produce more oils ?

- The lipid content of an algae species can be increased by imposing a stress factor, such as starving of nitrogen or silicon.
- In this manner, lipid fractions up to 70-85% have been reported for nitrogen starvation and 60% for silicon starvation

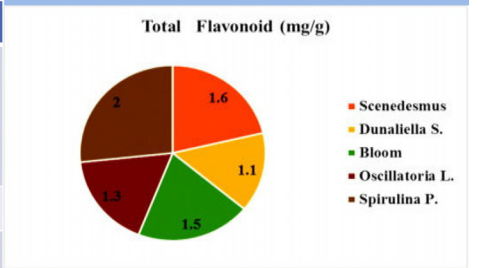
Algal Cell Harvest

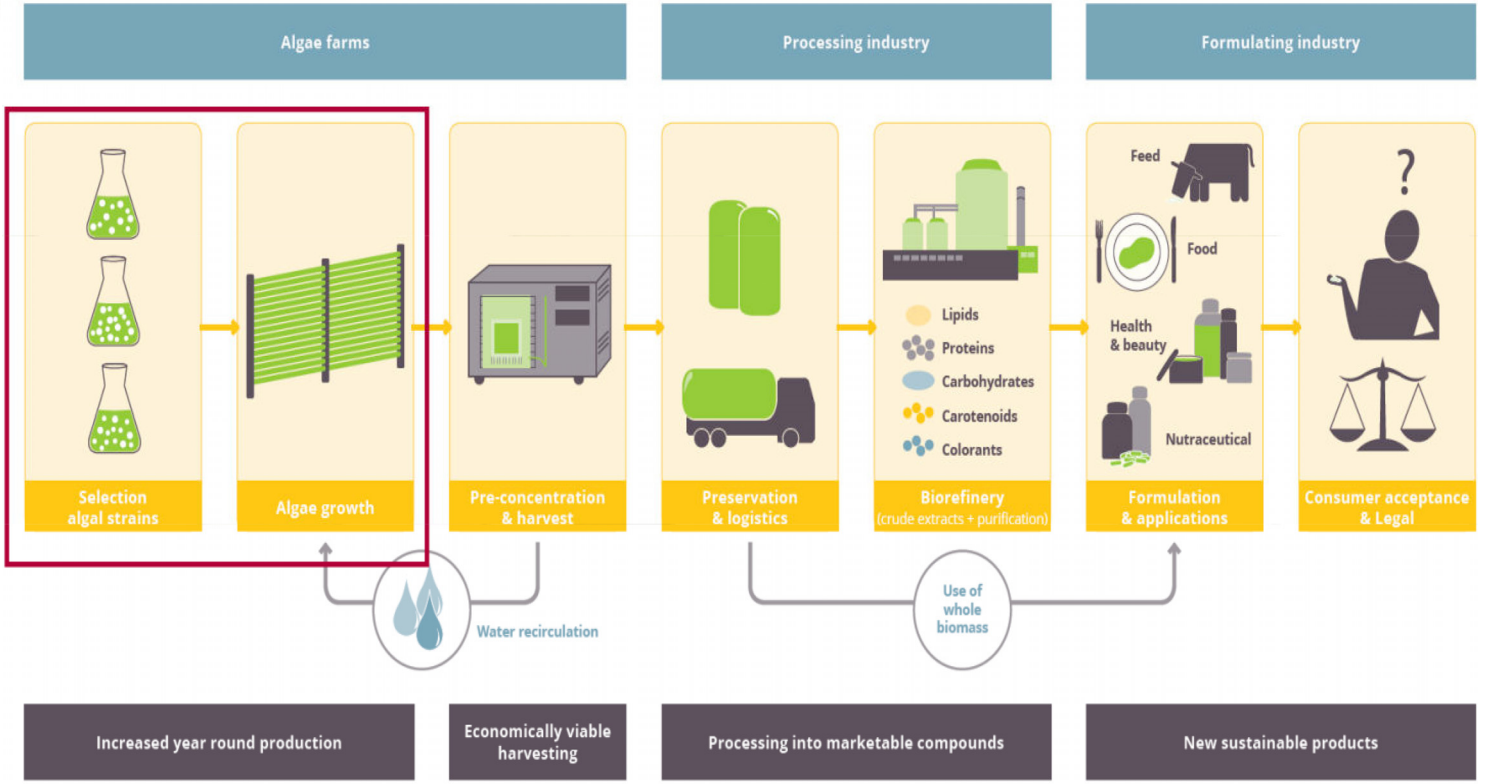
- Centrifugation- small microalgae
- Filtration- different size of algae
- Flocculation- Change of pH

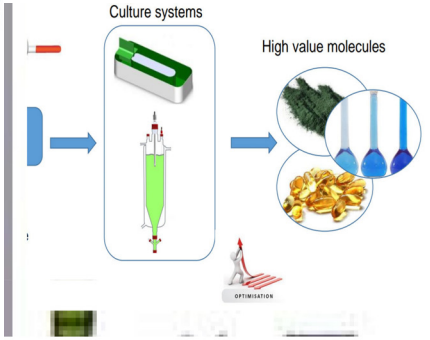
❖Acid flocculation – using alum and sulphuric acid
❖Alkali flocculation – using NaOH and KOH
❖Autoflocculation – Interrupting the CO_2 level



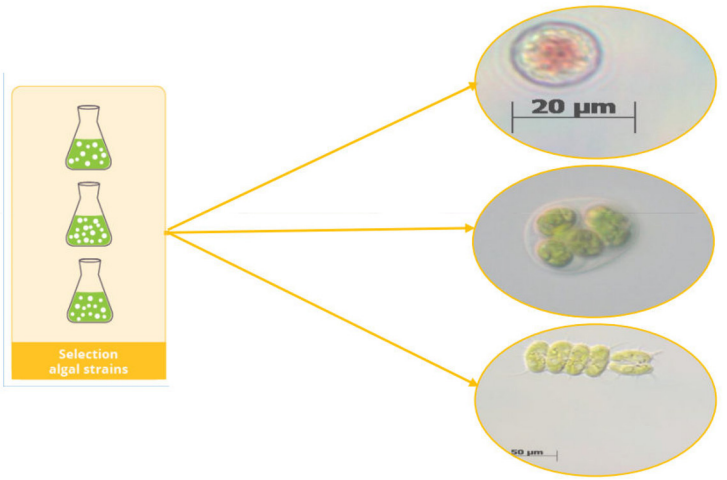
		20;5w3 EPA	Ew3HUFA	Protein%dw
ALGAE	CHAETOCEROS	13.6	16.4	24.4
	TETRASELMIS	4.7	5.2	49.8
	Spirulina			17.5
	Scenedesmus			21.0
Live feeds zoo	rotifers	2.5	3.1	62
	Artemia nauplii	2.8	3.7	55




Algae farms
Processing industry
Formulating industry
Selection algal strains
Algae growth
Pre-concentration & harvest
Preservation & logistics
Biorefinery (crude extracts + purification)
Formulation & applications
Consumer acceptance & Legal
Lipids
Proteins
Carbohydrates
Carotenoids
Colorants
Feed
Food
Health & beauty
Nutraceutical
Water recirculation
Use of whole biomass
Increased year round production
Economically viable harvesting
Processing into marketable compounds
New sustainable products



REED MARICULTURE USA



Porphyridium purpureum

THOMAS MORE

Chloromonas Typhlos

Chaire de Biotechnologie CentraleSupélec

Mixed culture: *Chlorella, Scenedesmus& Synechocystis*

JÜLICH FORSCHUNGSZENTRUM

Manufacturers can expand the market in several ways. The various end-use industries, such as cosmetics, agriculture, and pharmaceuticals, drive the global market. A few ways can expand the global market are:

- Increase Productivity: Manufacturers can expand the market by investing their million dollars in research and development activities. They offer affordable products to attract consumers by growing their microalgae-based production.
- Sustainable and Healthy Ingredients: Manufacturers are developing sustainable and eco-friendly microalgae rich in nutrient value. They also promote their sustainable products as a better health alternative to their consumers.
- Educate Customers: Many consumers are unknown about the benefits of microalgae. The manufacturers educate these consumers. Educating customers, raising awareness, and promoting microalgae-based products to increase sales volume.
- Collaborations: Manufacturers collaborate with several end-use industries to reach their relevant consumers. They develop advanced and sustainable products for various industries, such as food and beverages, cosmetics, etc.

In January 2022, Yemoja Ltd., a startup company that developed advanced ingredients from microalgae and further announced its plan to create red microalgae for plant-based steaks and burgers. Ounje, its new brand, resembles the red juices of animal meat without the requirement of artificial colors. The patented microalgal heme substitute congeals and browns like natural meat juices if cooked, apart from delivering the initial red pigment to plant-based meats.

In January 2022, Bharathidasan University based in Tamil Nadu signed a memorandum of understanding (MoU) with ZIGMA Global Environ Solutions for initiating a joint project worth Rs. 8.05 lakh on 'microalgae-based bioremediation of municipal solid waste leachate.' Algae production is a far more sustainable industry

than continuing to harvest 30% of the world fish catch for fishmeal and fish oil at ever increasing cost. The release in fishing pressure could have a dramatically favorable effect on marine ecosystems. The Global market would be ten times more profitable, If microalgae were used to replace fishmeal and fish oil globally, the effect would be to remove 30% of fishing pressure at the lower end of the food web and would contribute to a restoration of marine ecosystems

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CMFRI Training manual Ser. No.: 9/2016

Training Manual on Live Feed for Marine Finfish and Shellfish Culture

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Microalgae culture media and glass ware

Biji Xavier, Sekar Megarajan and Vamsi B

Introduction

Microalgae culture with desired species of flagellates or diatoms is the basis of any finfish or shell fish larval rearing and culture system. Natural sea water is a complex culture medium containing more elements and organic compounds, and which supports mixture of all phytoplankton and zooplankton. But in the laboratory culture, monospecies culture of micro algae is being maintained to support different culture requirements. Natural sea water with added nutrients is recommended, because direct sea water may not provide the optimum nutritional requirement of specific algae. So enrichment of natural sea water is necessary with the addition of macro nutrients, micro nutrients, trace elements and vitamins. Each micro alga needs the specific culture media, with basic nutrients like nitrogen, phosphorous, vitamins and trace metals for better growth and multiplication.

Importance of Culture Medium

Algal nutrient solutions or culture or growth medium are made up of mixture of chemical salts and water. The culture medium provides the material needed for the growth of algae. These nutrients solutions are formulated specifically for its use in aquatic environments and their consistency is more precise is for laboratory culture.The culture medium constituted with the addition n of macronutrients, micro nutrients and vitamins. Micro nutrients include nitrate, phosphate and silicate. Micro nutrients contain various trace metals. Vitamins like thiamin (B1), cyanocobalamin (B12) and sometimes biotin are commonly required for the growth of most of the micro algae.

Macronutrients (Nitrogen, Phosphorus and Silicon)

Nitrogen and phosphorus are the important macronutrients, for the growth and metabolism of algal cells, which are added to the culture medium as Nitrate ($NaNO_3$ /KNO_3) and phosphate (NaHPO4.H2O).. Nitrogen is key element for the formation of protein and nucleic acids accounts to 7-20% of micro algal cell dry weight. Phosphorous play its major role in the formation of energy carrier molecule (ATP) forms 1% dry weight of the algae. Algae requires inorganic carbon source in the form of CO2, carbonate or bicarbonate for its photosynthesis.Silicate is necessary for the cell wall development of diatoms and is added in sodium silicate form (Na_2SiO_3 $9H_2O$) to the culture medium.

Micronutrients (Fe, Mn, Co, Zn, Cu and Ni)

Micro nutrients are trace metals which are present in algal cells in extremely small quantities (<4ppm), which are essential for the physiological growth of algae. Iron (Fe,) Manganese (Mn), Cobalt (Co), Zinc (Zn), Copper (Cu) and Nickel (Ni) are the most important trace metals required by algae for the various metabolic functions. Deficiencies in trace metals may lead to the slow algal growth and excess concentration may inhibit the growth, impair photosynthesis and finally damage the cell membrane of the algae. Typical trace metal stock solutions may consist of chloride or sulphate salts of zinc, cobalt, manganese, selenium, and nickel, and they are kept in a solution containing the chelator EDTA. Iron is an important trace metal required for the algae for its normal growth, photosynthesis and respiration. Iron is usually kept as a separate solution, may be added as ferric chloride or ferrous sulphate. EDTA is used as chelator and is available as disodium salt ($Na_2EDTA.2H_2O$) that is readily soluble in water.

Vitamins

Vitamins are organic micronutrients, which are essential for photosynthetic microalgae. Algal species require different combination of vitamins, mostly Vitamin B_{12} (Cyanocobalamin), Vitamin B_1(Thiamine) and Vitamin B_7 (Biotin). The general order of

vitamin requirements for algae is vitamin B_{12} > thiamine > biotin. Vitamins are normally added aseptically (through a 0.22-mm filter) after the medium has been autoclaved.

Some key aspects in medium preparation

In general, chemicals required for the microalgal culture media preparation are available from various chemical suppliers. Reagent grade salts from Merck showed good performance in the algal culture. The organic chemicals such as vitamins, buffer and chelators available from Sigma Chemical Company is better to use compared to others. Whenever the shortage of chemicals from the particular company occurs that also need to be taken care of seriously. Nutrients come with different salts and hydration, (copper and zinc) may be available as $CuSO_4$ or $CuCl_2$ and $ZnSO_4$ or $ZnCl_2$. Some nutrients also come with different hydrations (NH_2O). Substituting one form wth other due unavailability or shortage also may lead to poor growth or no growth of micro algae. Thus the change in chemical form and also different hydaration can lead to precipitation problems of the salts in the culture medium. Therefore the chemicals with correct form and correct recipes only will lead to a successful micro algal culture.

Stock solutions

Stock solutions are made with accurate weighing of the chemicals in the specific culture media, dissolved in the specific volume of distilled water. Some chemicals (EDTA) may need heat treatment to dissolve completely in the water, otherwise which may lead to unnecessary precipitation of the nutrients in the medium. But, vitamin stocks should be prepared with normal distilled water and should not be exposed to any heat treatments. The vitamin stokes are advised to keep in dark bottles. There are two terms used in stock preparation, as working stock and primary stocks. Working stocks are the small quantity (aliquot) of solution which are directly used for the preparation of final medium and Primary stocks are formed from several single substance solution and finally combined to form the working stock.

Water sources, treatment and storage

Successful micro algal culture needs good quality natural sea water free from pollution. The enrichment of the sea water can be done for specific algal species with the addition of nutrients trace metals and vitamins. The sea water from off shore area can be passed through slow sand filter in order to remove turbidity and pathogenic organisms through various biological and chemical processes. Furthur, the dissolved organic matter can be removed with high intensity ultra violet light. The UV sterilized water is stored and used for the regular micralgal culture. Furthur, chemical sterilization of the sea water using autoclave is practiced for the micro algal culture. Sometimes salinity of the sea water also needs to be adjusted with specific algal species. In general, sea water salinity varies from 32-35ppt and most of the algae grow with that salinity. But some algae species require low salinity; in that case salinity must be decreased by adding deionised water before the addition of any nutrients, trace metals to avoid dilution of these compounds.

Culture media recipes

The selection of culture media mainly depend on the type algae species cultured. Diatoms like *chaetoceros, skeletonema, Thalassiosera, tetraselmis* etc., need silicates for the formation of silicious cell wall in addition to nitrate, phosphate, trace metals and vitamins . Diatoms and nanoplankters performed better growth with Media like Erd-Schreiber's (Table 1)and Miquel's media (Miquel, 1892) (Table 2). Schreiber’s medium (modified serial dilution culture method) also available with the addition of some chaelators and vitamins along with basic Schreiber’s medium for the various micro algal culture.

Table: 1 Composition of Schreiber's medium

Potassium nitrate	0.1g
Sodium orthophosphate	0.02g
Soil Extract	50ml
Filtered and sterilized seawater	1L.

Soil extract is prepared by boiling garden soil (1kg in 1L freshwater) for one hour. Keep it overnight and decant the clear water and kept it in a bottle. 50ml of this extract is added to each litre of sterilized sea water. This media can be used as medium for isolating the micro algae.

Table 2. Composition of Miquel's medium

A	Pottassium Nitrate	20.2g
	Distilled water	100ml
B	Sodium orthophosphate	4g
	Calcium Chloride	2g
	Ferric Chloride	2g
	Hydrochloric acid	2ml
	Distilled Water	100ml

Table 3. Schreiber's medium (modified serial dilution culture method)

Potassium nitrate (5g in 100ml of DW)	0.25ml
Sodium orthophosphate (1g in 100ml)	0.25ml
EDTA (1.2g in 100ml	0.15ml
Vitamin Mixture (Thiamine,-200mg Biotin-1mg, Cyanocobalamin 1mg in 1L DW)	0.50ml
Soil extract	3ml
Sterilized seawater	250ml

The medium is autoclaved at 800C for 15 minutes, then cooled down to room temperature in running water. Vitamin mixture should be added after cooling the medium.

Most of the culture media for micro algal culture are composed of chemicals, trace metals and vitamins. Most commonly used culture media used for stock culture and mass culture of micro algae in the laboratory is **'Conway' or 'Walne's medium**

(Walne,1974) (Table 4). Mainly used for indoor culture of *Nannocloro*psis, *Chlorella, Diatoms like Chaetoceros, Skeletonema, halassiosera,Tetraselmis.*

Table 4. Composition of Conway / Walne's medium:

Solution (A)		
1.	Potassium Nitrate (KNO_3)	100g
2.	Sodium di-hydrogen orthophosphate ($NaH_2PO_4.2H_2O$)	20g
3.	EDTA di-sodium salt (Na_2EDTA)	45g
4.	Boric Acid (H_3BO_3)	33.4g
5.	Ferric Chloride ($FeCl_3$)	1.3g
6.	Manganous Chloride ($MnCl_2.2H_2O$)	0.36g
7.	Distilled Water	1L
Solution (B)		
1.	Zinc Chloride ($ZnCl_2$)	4.2g
2.	Cobalt Chloride ($CoCl_2.6H_2O$)	4.0g
3.	Copper Sulphate ($CuSO_4.5H_2O$)	4.0g
4.	Ammonium molybdate ($(NH_4)_6Mo_7O_{24}.4H_2O$)	1.8g
5.	Distilled Water	1L
6.	Concentrated HCl	
Solution (C)		
1.	Vitamin B_1 (Thiamine)	2g
2.	Vitamin B_{12} (Cyanocobalamin)	100mg
3.	Distilled Water	1L
Solution (D)		
1.	Sodium silicate ($Na_2SiO_3.5H_2O$)	40ml
2.	Distilled water	1L

Stock culture: only autoclaved seawater should be used.

Working Solution for mass culture: Add 1ml of each Solution A, 0.5ml of B, 0.1 ml of C and 1ml of D into 1 L sea water. D: Only for Diatoms (*Chaetoceros*)

Fig. 1. Conway medium

F/2 medium (Guillard, R. R. and Ryther, J. H. 1962) is widely used enriched seawater medium designed for growing marine algae culture. Commonly used for the indoor culture of *Isochrysis* and outdoor culture for the *Nannocloro*psis, *Chlorella*, *Diatoms* like *Chaetoceros, Skeletonema,Thalassiosera,Tetraselmis etc.*

Fig. 2. F/2 medium

Table 5. Composition of Guillard's F/2 media used for micro algal culture

Solution (A)			
1	Sodium nitrate ($NaNO_3$)	75g	1 L distilled water
2.	Sodium phosphate ($NaH_2PO_4.H_2O$)	5g	
P.S.Solution*			
1	Copper Sulphate ($CuSO_4.5H_2O$)	10g	1 L distilled water
2	Zinc Sulphate ($ZnSO_4.7H_2O$)	22g	1 L distilled water
3	Cobalt Chloride($CoCl_2.6H_2O$)	10g	1 L distilled water
4	Manganous Chloride ($MnCl_2.4H_2O$)	180g	1 L distilled water
5	Sodium Molybdate ($Na_2MoO_4.2H_2O$)	6g	1 L distilled water
Prepare each solution separately in 1L bottles			
Solution (B)**			
1	EDTA di-sodium salt (Na_2EDTA)	4.36g	1 L distilled water
2	Ferric Chloride ($FeCl_3.6H_2O$)	3.15g	
Add 1ml of each solution (P.S solution1-5) each to 1 L of EDTA & $FeCl_3$ mixed solution			
Solution (C)***			
1	Thiamin HCl	20g	1 L distilled water
2	Biotin	100mg	1 L distilled water
3	Cyanocobalamine (B_{12})	100mg	1 L distilled water
Add 5ml of each solution into 1L of sea water			
Solution (D)****			
1	Sodium Silicate ($Na_2SiO_3.9H_2O$)	35g	1 L distilled water
Stock culture: only autoclaved seawater should be used. *P.S. Solution: Each solution 1L should be prepared separately in different bottles **Solution B: 1L of EDTA & $FeCl_3$ mixed solution with P.S. solution (1ml of each) *** Solution C: 1L of sea water with Thiamin, Biotin and Cyanacobalamin solution (5ml each) **** Solution D: only for Diatoms (*Chaetoceros*)			
Working Solution for mass culture: Add 1ml of each Solution A, I C and D into 1 L sea water. D: Only for *Chaetoceros*			

For the mass culture of micro algae media named TMRL and PM (Gopinathan, 1982), is reported to be effective. So many media are available to culture the algae, but the exact requiremtnt during each growth stages need to be studied in detail.

Table 6. Composition of TMRL medium 100ml (Tung Kang Marine Res. Lab)

Potassium nitrate	10g
Sodium orthophosphate	1g
Ferric Chloride	0.3g
Sodium Silicate	0.1g

Glass wares used in media preparation

Reagent bottles (250 ml, 500 ml, 1000 ml & 2000 ml), culture tubes/test tubes (20 ml), conical flasks (100 ml, 250 ml, 2000 ml & 3000 ml), Haufkin culture flasks (3000 ml & 4000 ml). etc. In general Borosilicate glass ware should be used exclusively for all glassware, including stock bottles, beakers, test tubes and flasks. Teflon or plastics wares are also recommended, because they will reduce the breakage. Manufacturer's specifications for the particular glass ware usage such as storage for concentrated solutions and autoclaving also need to be considered. The glass wares and plastic wares used for culture medium preparation should keep separately from general purpose laboratory use. New glass wares and plastic wares need to be degreased with dilute NaOH, soaked in dilute HCL and then soaked in deionised water for several days before use. Glass wares should be autoclaved and cleaned glass wares and plastic wares should be stored in closed cupboards, and open vessels should be covered. Tubing used to siphon water from one bottle to another also should be cleaned properly. All containers used for culture and media stocks should be carefully selected to avoid toxic compounds. For general culture purpose, borosilicate glass wares and tissue culture grade polycarbonate or poly propylene plastic wares are recommended. Other accessories include,

micropipette, dropper with teats, tissue paper, copper wire /inoculation loop, spirit lamp, aluminum foil etc.

Cleaning of glass wares

The glass wares for the isolation, laboratory culture, maintenance and mass culture should be cleaned thoroughly prior to sterilization. The glass wares used for the indoor culture need to be cleaned with chromic acid. For outdoor culture of micro algae, the carboys used are cleaned by common salt and rinsing with tap water 4-5 times and keep for sun drying.

Preparation of Chromic acid:

5g of Potassium chromate partly dissolved in 5ml distilled water. Add 100 ml of Conc. H_2SO_4 with stirring and cooling and maintain as stock solution.

Chromic acid working solution:

Add 500 ml of the stock solution to 100 L of tap water.

Fig. 3. Cleaning of glass wares

The cleaning procedure is:

1. Rinse the glass wares with tap water.
2. Clean with brush and rinse again with tap water
3. Fill the glass wares with chromic acid (working solution) and keep it for minimum1 hr

4. Empty the Glass wares and rinse it with tap water 3-4 times
5. Drain out the water and keep the glass ware in the oven for drying.

Equipments in Micro algal culture

Analytical and top-loading balances	To weigh the chemicals accurately
p^H meter	To check the p^H of the culture media
Hot plate magnetic stirrer	To dissolve the chemicals in media completely
UV Filtration Unit	UV Filtered sea water is pre requisite for all micro algal culture.
Autoclave	Sterilization of UV filtered sea water using autoclave is necessary to prevent further contamination in the growing algal culture.
Hot Air Oven	Cleaned glassware and accessories for the inoculation of micro algae need to be sterilized with dry heat generated from Hot air oven.
Microscope	Identification of the micro-algae as well as for the determination of cell concentration of the culture, a good microscope is required. Since the flagellates are identified by noting the number of flagellae and other cell characteristics, a powerful microscope is advisable.
Haemocytometer	Counting chamber used to determine the cell counts in the micro algal culture.
Camera	Photographs of the cells can be documented

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"Live Feed for Marine Finfish and Shell fish Culture"

17th - 22nd March 2016

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