AlgaeCeuticals

Development of microalgae-based natural UV Sunscreens and Proteins as cosmeceuticals and nutraceuticals

Microalgae cultures in laboratory conditions

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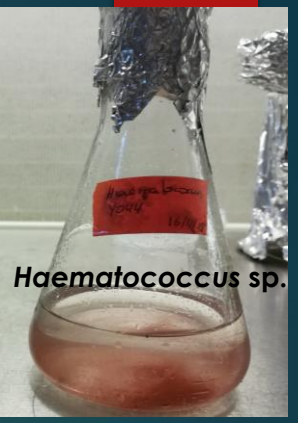
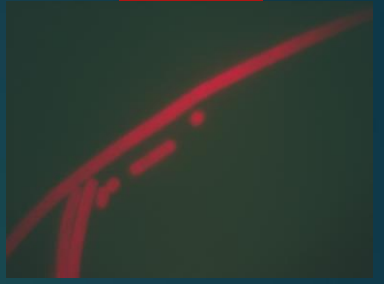
AlgaeCeuticals – the project



"Microalgae represent a largely untapped reservoir of novel and valuable bioactive compounds".

"The biological and chemical diversity of the microalgae, has great potential for industrial development as pharmaceuticals, cosmetics and nutritional supplements".

Microalgae



Microalgae are microscopic algae found in aquatic habitats that have the ability to **photosynthesize**

- For an entirely autotrophic alga the following are essential:

light + CO_2 + H_2O + nutrients + trace elements

↓

Photosynthesis

↓

synthesizing all the biochemical compounds necessary for growth

Auxotrophy: Only a minority of alga seem, however, to be entirely autotrophic; many are unable to synthesize certain biochemical compounds (certain vitamins, for example) and will require these to be present in the medium

Microalgae

Microalgae are found everywhere!

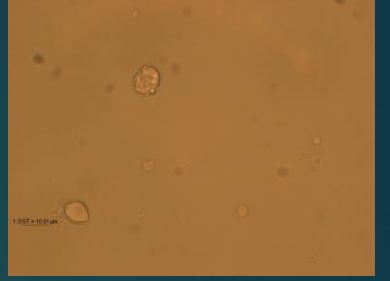
Can be extracted from the environment through a process known as **culture isolation/"fishing"** the strain:

- separating the strain from the surrounding organisms
- placing the strain into a suitable medium and environment to promote growth

The aim is to obtain an **axenic** (no other microorganisms) culture or possibly a **monoalgal** culture

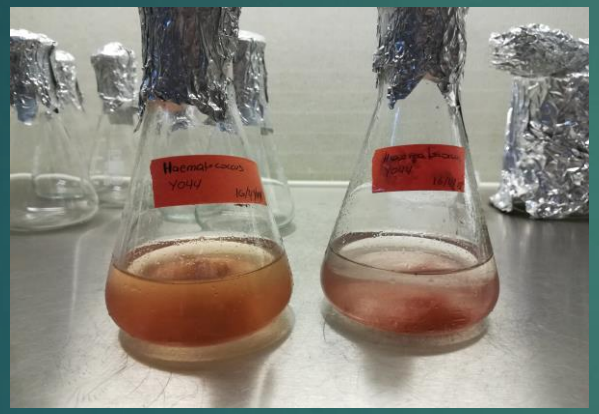
Isolation process is complex and involves identifying the strain

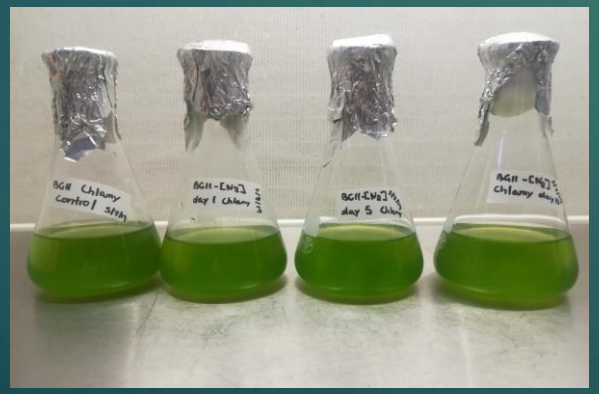
Cultivating microalgae



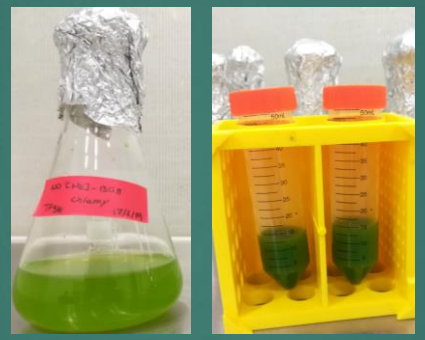
Culture conditions should resemble the alga's natural environment

Liquid cultures





Flasks



Plates

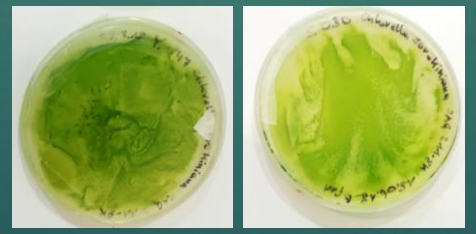
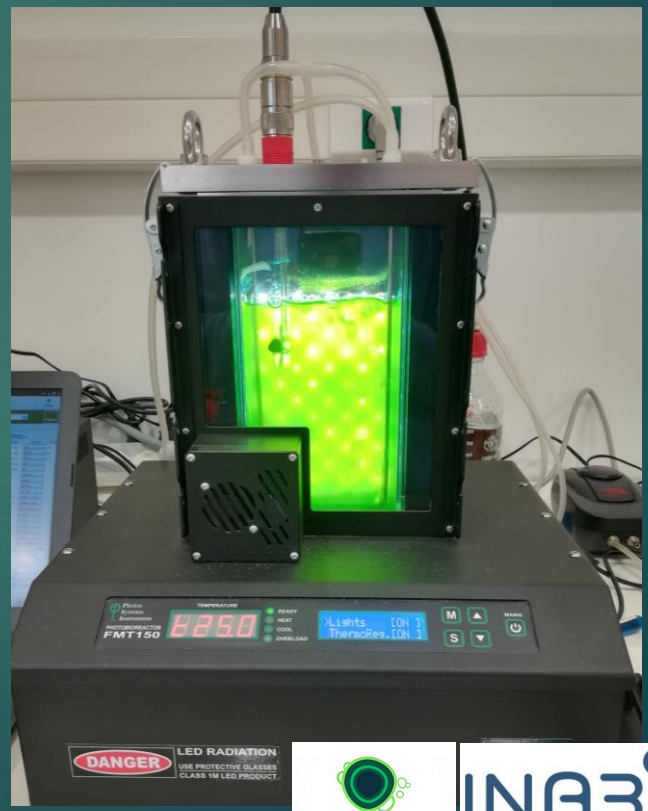
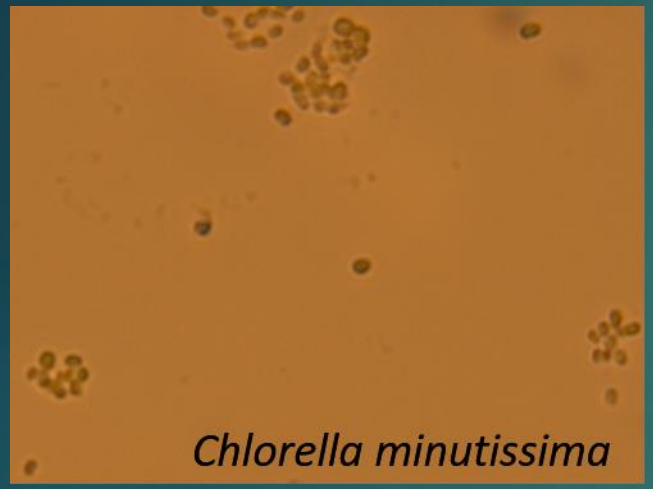
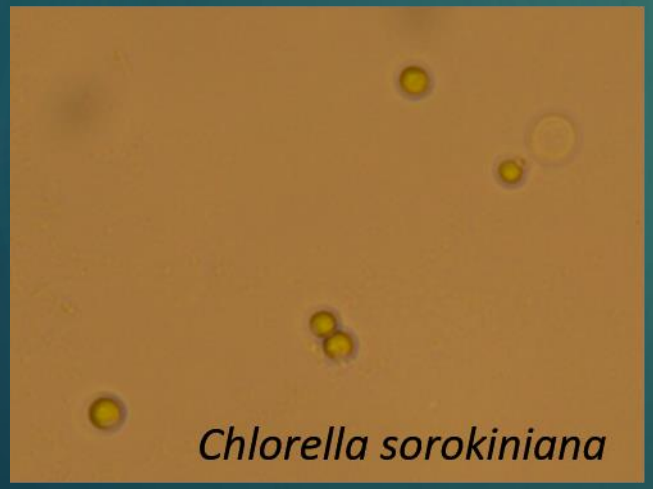


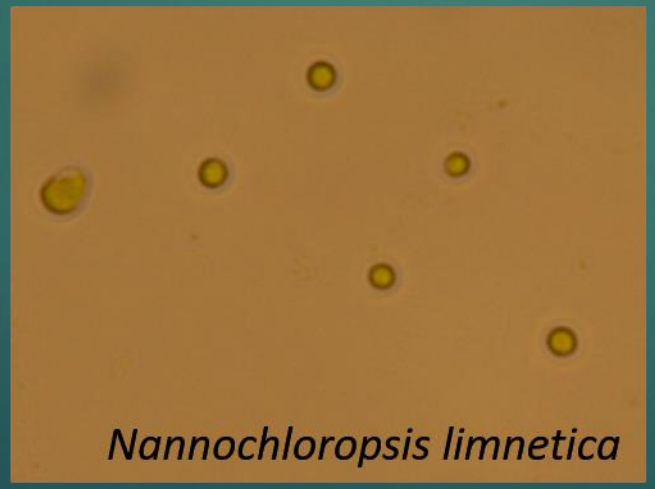
Photo Bioreactor



Cultivating microalgae







Cultivating microalgae

There are 2 kinds of cultures

- **In limited volume cultures (batch)**
  - **Subculturing**, i.e. transferring a small volume of existing culture to a large volume of fresh culture medium at regular intervals
- **In continuous cultures**
  - A volume of fresh culture medium is added automatically at a rate proportional to the growth rate of the alga, while an equal volume of culture is removed

growth occurs according to a sigmoid curve

Culture components

In their natural habitats microalgae obtain all the required nutrients, minerals and vitamins from the water in which they live!

! To grow them in the lab we need growth media !

There are 3 distinct components:

- ✓ **The culture medium (liquid or solid)**

  sources of: nitrogen (in the from of nitrate, nitrite and ammonia), phosphorus, vitamins and trace metals along with dissolved inorganic C

- ✓ **The algal cells**
- ✓ **Air to allow exchange of carbon dioxide between medium and atmosphere**

Physical parameters

Temperature

Ideally as close as possible to the temperature at which the organisms were collected

Light

- Natural light (sufficient to maintain cultures in the laboratory)
- Cultures should never be exposed to direct sunlight → may cause photopigment damage
- Artificial lighting by fluorescent bulbs for culture maintenance and experimental purposes

Many microalgal species do not grow well under constant illumination, and hence a light/dark (LD) cycle is used (maximum 16:8 LD, usually 14:10 or 12:12)

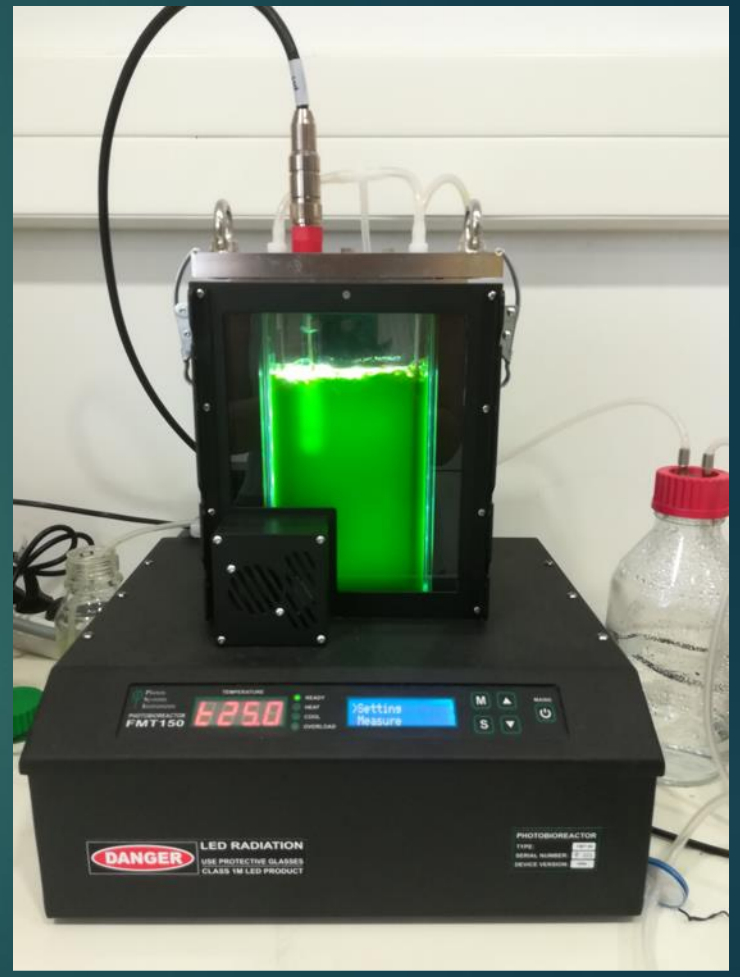
Wahidin, S., Idris, A., & Shaleh, S. R. M. (2013). *The influence of light intensity and photoperiod on the growth and lipid content of microalgae Nannochloropsis sp. Bioresource Technology, 129, 7–11.* doi:10.1016/j.biortech.2012.11.032

Physical parameters

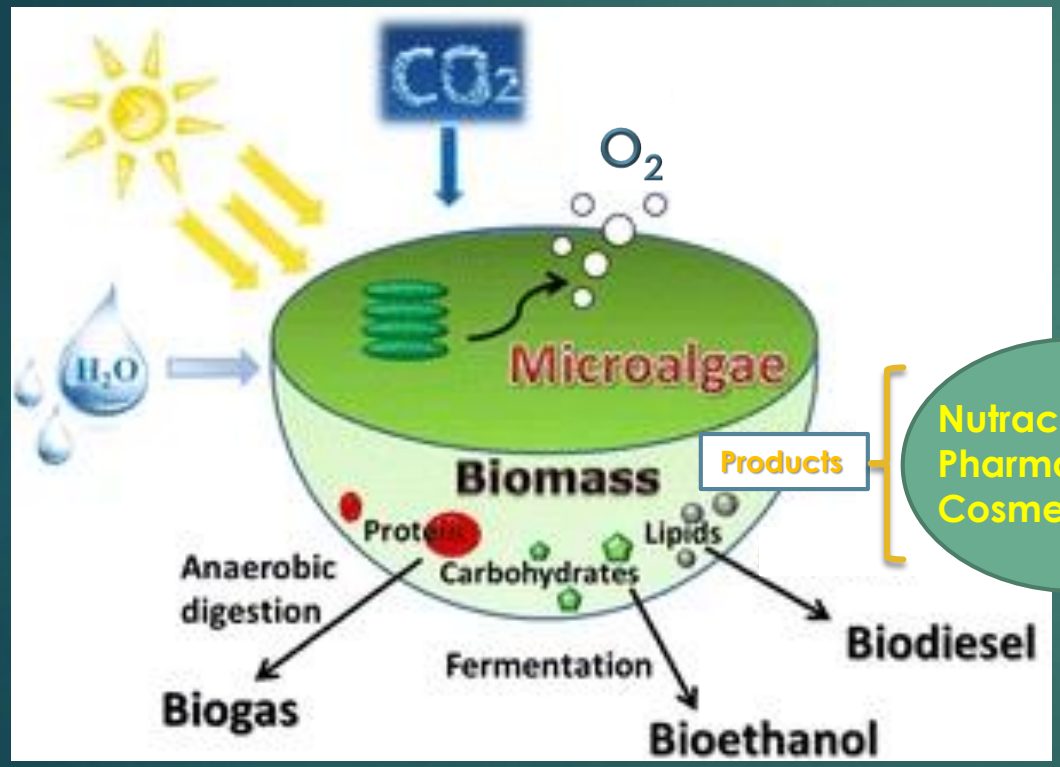
Gentle Mixing

- when cells must be kept in suspension in order to grow
- in concentrated cultures to prevent nutrient limitation effects due to stacking of cells and to increase gas diffusion
- bubbling with air (may damage cells)
- gentle manual swirling

Most cultures do well without mixing, particularly when not too concentrated, but when possible gentle manual swirling (once each day) is recommended!



Production chain of microalgae biomass



Microalgae are micro-factories

The accumulation of biomass requires sunlight, water, nutrients and CO_2

Modified image from Wang, J., Yin, Y. Fermentative hydrogen production using pretreated microalgal biomass as feedstock. *Microb Cell Fact* **17,** 22 (2018) doi:10.1186/s12934-018-0871-5

METHODS

1. Preparation of media (for liquid and solid cultures)
2. Autoclave all conical flasks and equipment to be used
3. Work under sterile conditions in a Laminar flow hood

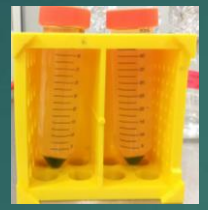
Microalgae cultures can be preserved as follows:

Liquid	→	**Solid**
Solid	→	**Solid**
Solid	→	**Liquid**
Liquid	→	**Liquid**

METHODS

Liquid to Liquid

Concentrated culture



Diluted start culture in freshly prepared medium

Culture after 2 weeks

Liquid to Solid

Take 100 μL of a dense culture and plate it with a drigalski spatula after sterilizing it with pure EtOH and flame

Solid to Solid

Sterile Eppendorf tubes → add 200 μL of the liquid medium → use the loop to take a few colonies → dissolve them in the Effendorf tube → use 100 μL of that culture → use a drigalski spatula to plate