AquaVIP Klaipeda - Innovative Aquaculture Summer School
Klaipeda University – Klaipeda Science & Technology Park

Blue Biotechnology Pipeline: From Discovery to Application

Tuesday, June 29th 2021

Microalgae & Fish Waste

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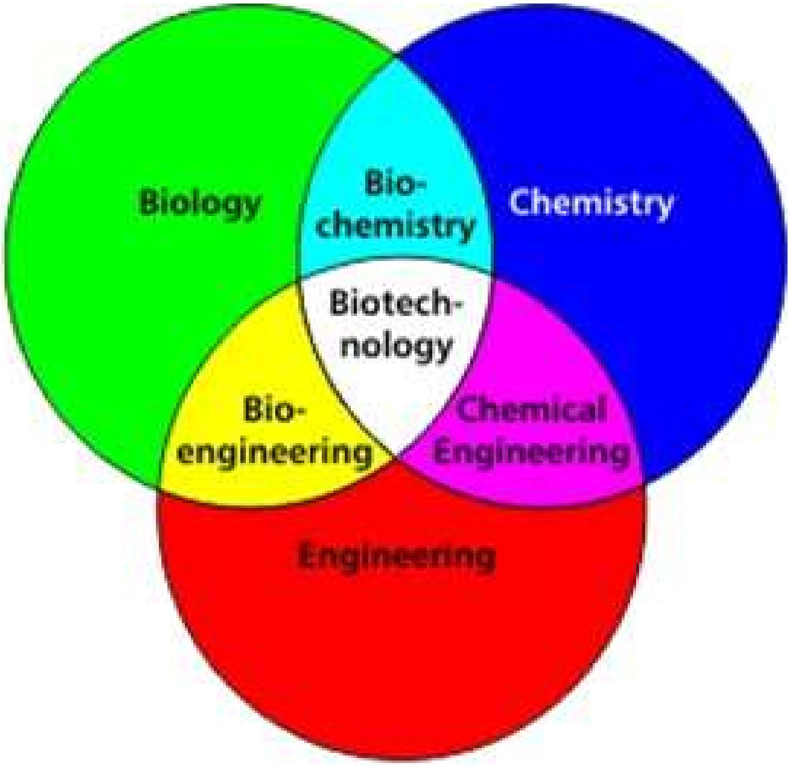
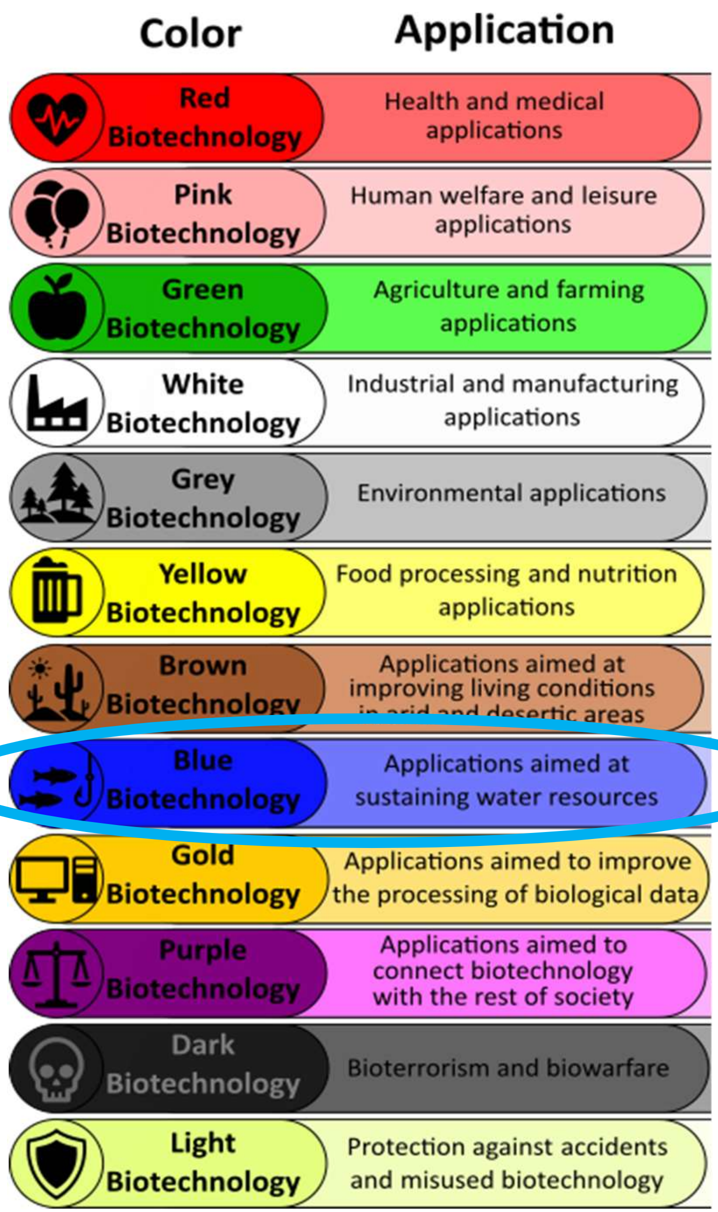
Jerónimo Chirivella Martorell PhD.



- Biotechnology:
  - technology that utilizes biological systems, living organisms or parts of them to develop or create different products and services.



- Blue Biotechnology:
  - concerned with the exploration and exploitation of the resulting diverse marine organisms in order to develop new products and services.
  - One of 5 pillars of Blue Growth Strategy of EC ➔ **Sustainable Blue Bioeconomy**

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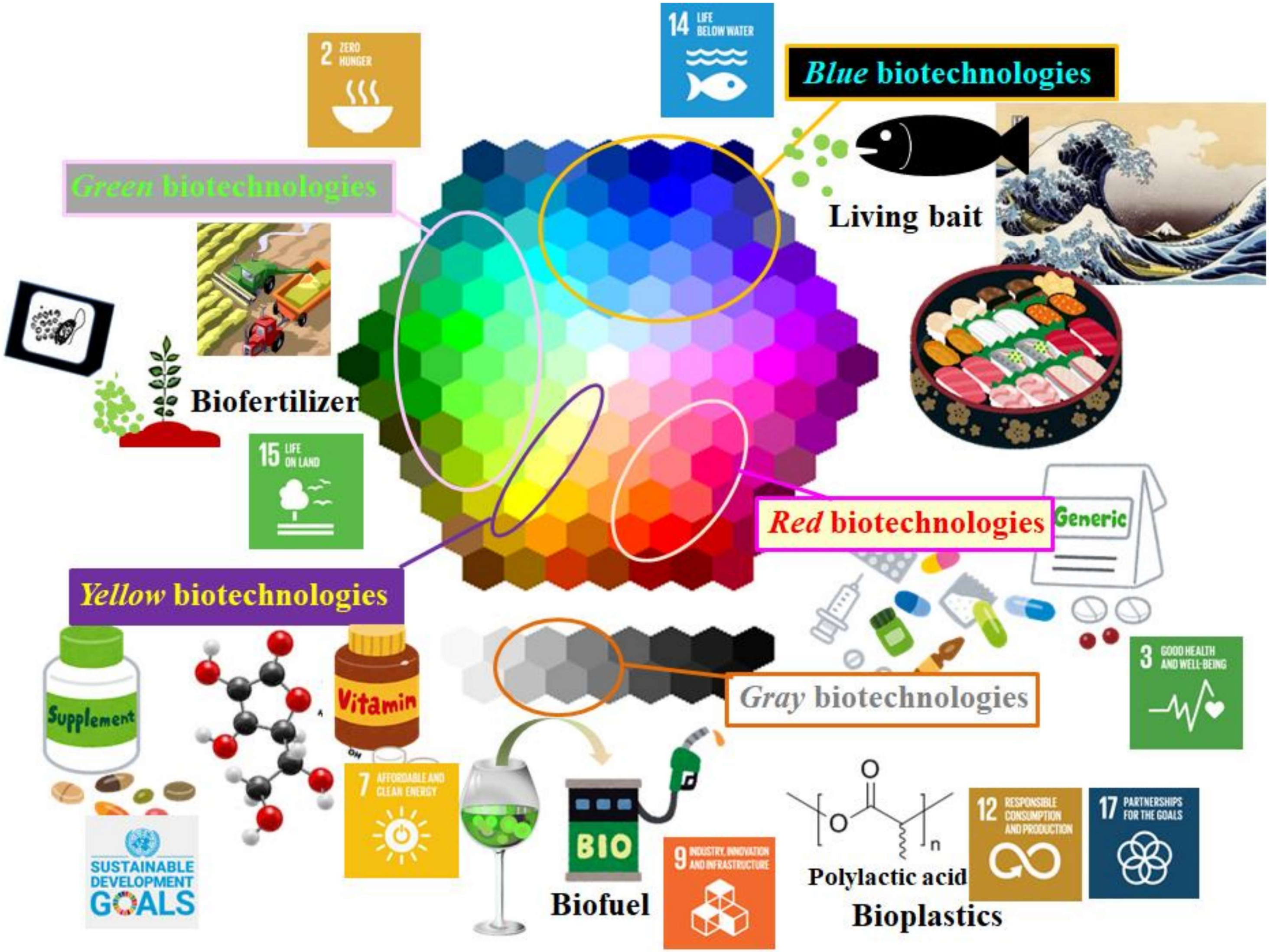
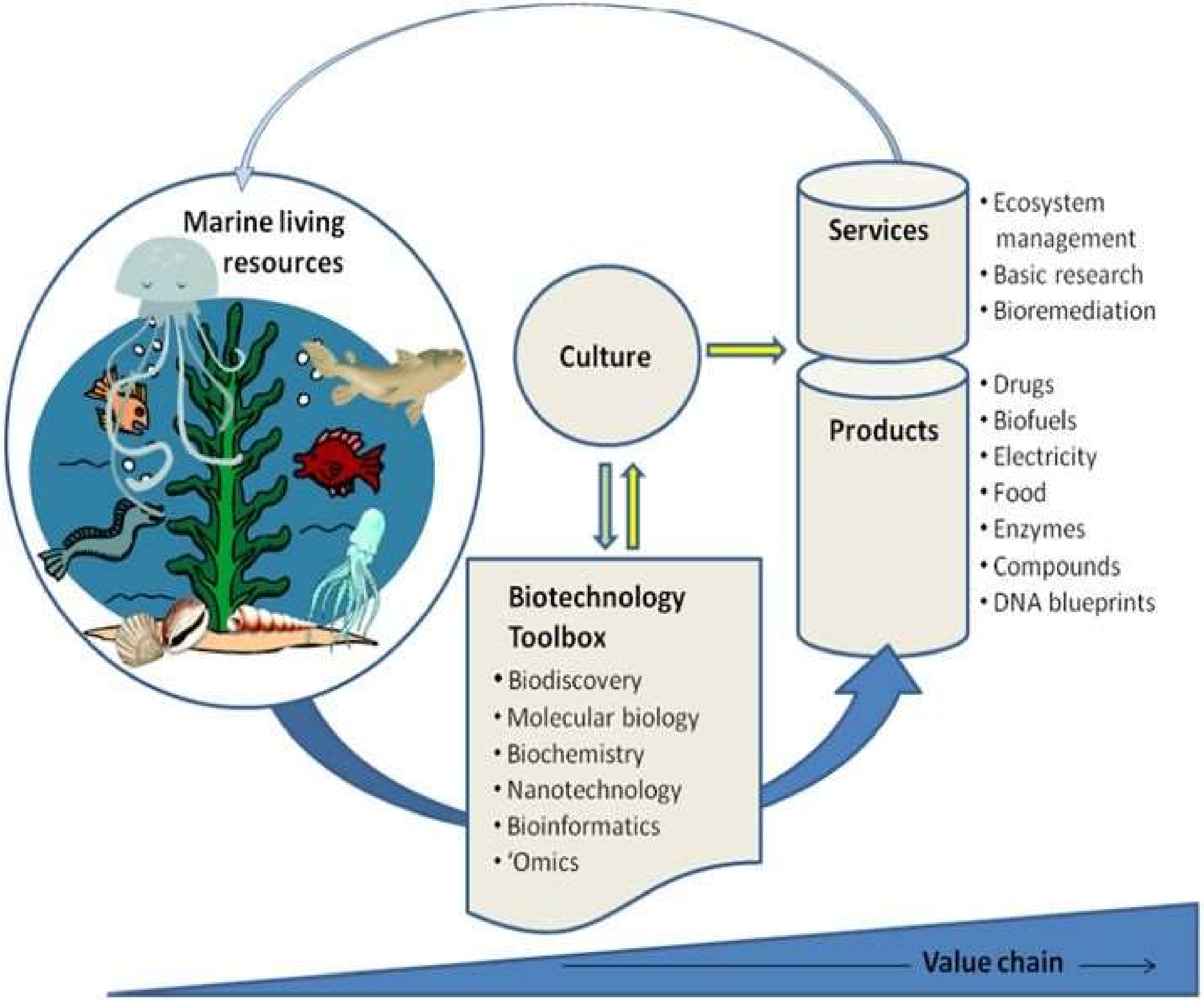


- Relationship between the different colors of the rainbow color code of biotechnology and **microalgal** potentials as food, feed, and feedstocks for biorefinery. Several panels with label numbers are logos of Sustainable Development Goals.

- Blue biotechnology pipeline



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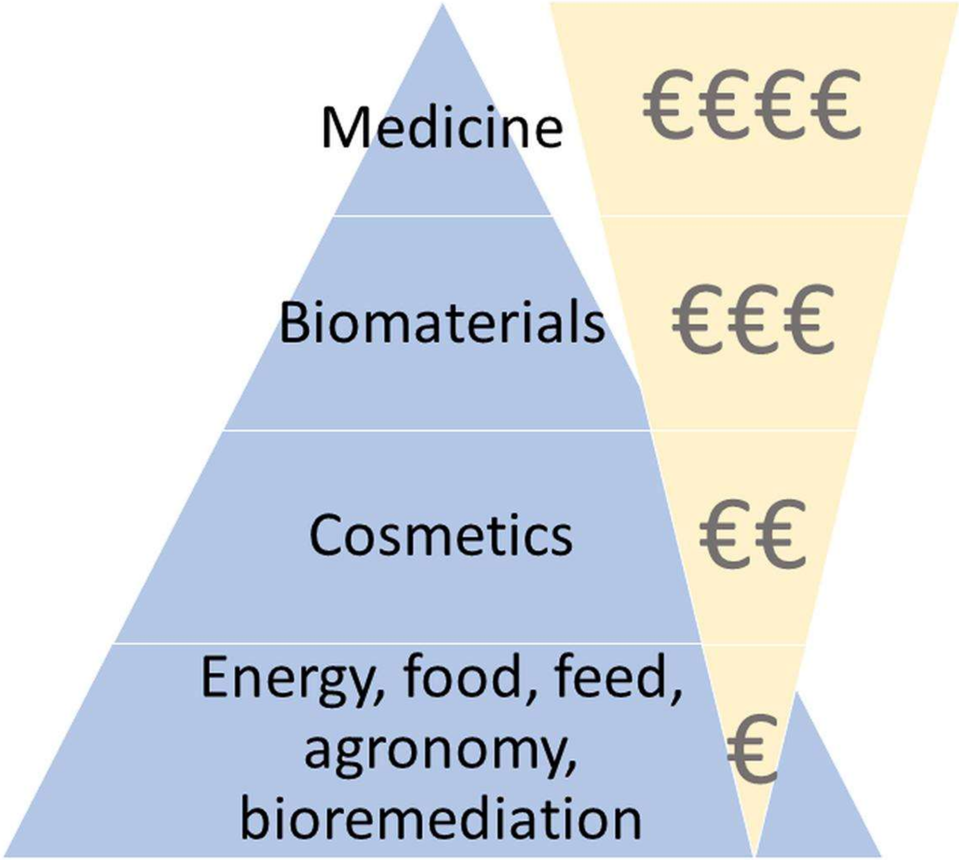
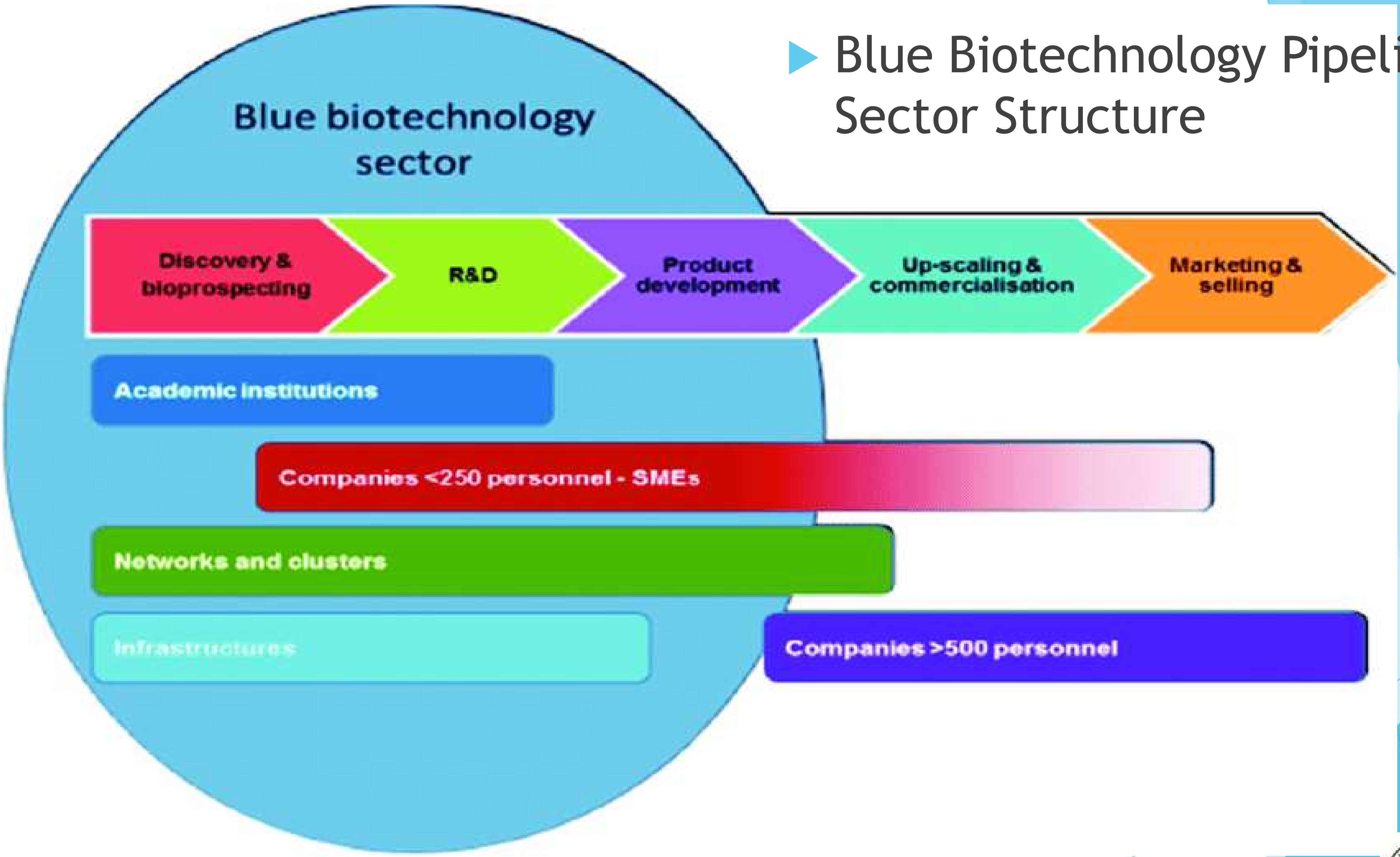


- Blue Biotechnology products pyramid value

Blue Biotechnology Pipeline & Sector Structure



Discovery: why should we look for into the sea?

- Marine organisms are rich in bioactive metabolites and their natural products are often of new chemical structure and usually different from their terrestrial counterparts.

- The unique properties of marine organisms are the product of their exclusively marine life and the effect exerted by the characteristics of seawater on their chemistry-physiology and metabolism (in addition to other adaptations, eg: flexibility of algae in the intertidal zone against the rigidity of the plants, development of gas vesicles or vacuoles to guarantee their buoyancy and light capture).

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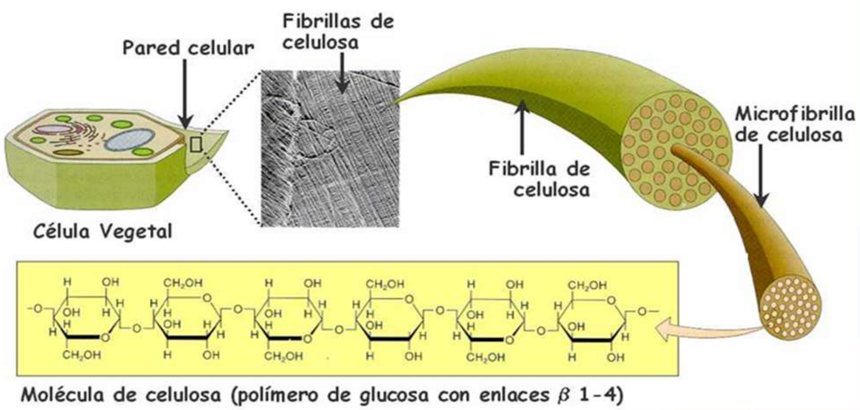
Discovery: why should we look for into the sea?

- Rigid terrestrial plant due to the composition of its cell wall

- flexible marine plants due to the composition of its cell wall that also has other properties ...



Presence of polyanionic polysaccharides in the cell wall. - The sulfate and carboxyl groups of the cell wall (anionic groups) act as the main metal ion scavenging complexes.

When there is a high concentration of metal ions in the aquatic environment, the cell wall of the algae prevents their entry into the cytoplasm, acting as an exclusion mechanism, an ionic barrier.

At low concentrations of ions in the medium, the high capacity of the cell wall of brown algae in ion exchange was demonstrated, which is 3.5 times higher than in terrestrial plants.

Discovery: why should we look for into the sea?

- Only prokaryotic and eukaryotic microorganisms comprise a vast and undocumented existing biological diversity. A phylogenetic, biochemical and physiological diversity that far exceeds that found in macro-organisms.
- However, current research suggests that less than 1% of the total diversity of marine microorganism species can be cultured by commonly used methods.
- This means that the application potential of the compounds produced by 99% of marine microorganisms is still unknown.
- However, one of the main limitations of the development of marine bioproducts is the availability of biological material. The natural abundance of organisms would not support development based on natural harvesting.
- Some options for the sustainable use of marine resources are: chemical synthesis, aquaculture of the organism of interest, cell culture, molecular cloning and biosynthesis. Options that require knowing the fundamental biochemical pathways by which bioproducts are synthesized.

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Discovering the chemodiversity

- **Natural product: any well-defined organic molecule produced by a living organism and not restricted to a secondary metabolite**

- **Primary metabolites** are essential for the life and reproduction of cells

- **Secondary metabolites** are only found accidentally and are not essential for the life or survival of the organism that produces them, although they can confer adaptive advantages to the species for their survival in the biological community and environment.
  - Secondary metabolism can be demonstrated genetically, biochemically or physiologically.

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Discovering the chemodiversity

MAIN CHARACTERISTICS OF SECONDARY METABOLITES:

- Can be found in any group of organisms without distinction (sometimes they are formed by relatively small groups of organisms of specific taxonomy, at other times by specific strains but not by taxonomically defined species).
- Smaller and chemically more diverse than the primary metabolites (proteins, lipids, nucleic acids, carbohydrates).
- Not essential for growth and reproduction, a significant number have no apparent biological function
- Generated by branched pathways of primary metabolism.
- Generated as a result of defense mechanisms and / or excessive metabolic production.
- Confer selective and adaptive advantage to organisms that produce them
- Usually inverse correlation between specific growth rate and formation of secondary metabolites. (Generation of metabolites under stress conditions of the organism)
- Production extremely dependent on environmental conditions ➔ Production can be regulated by the cultivation conditions.

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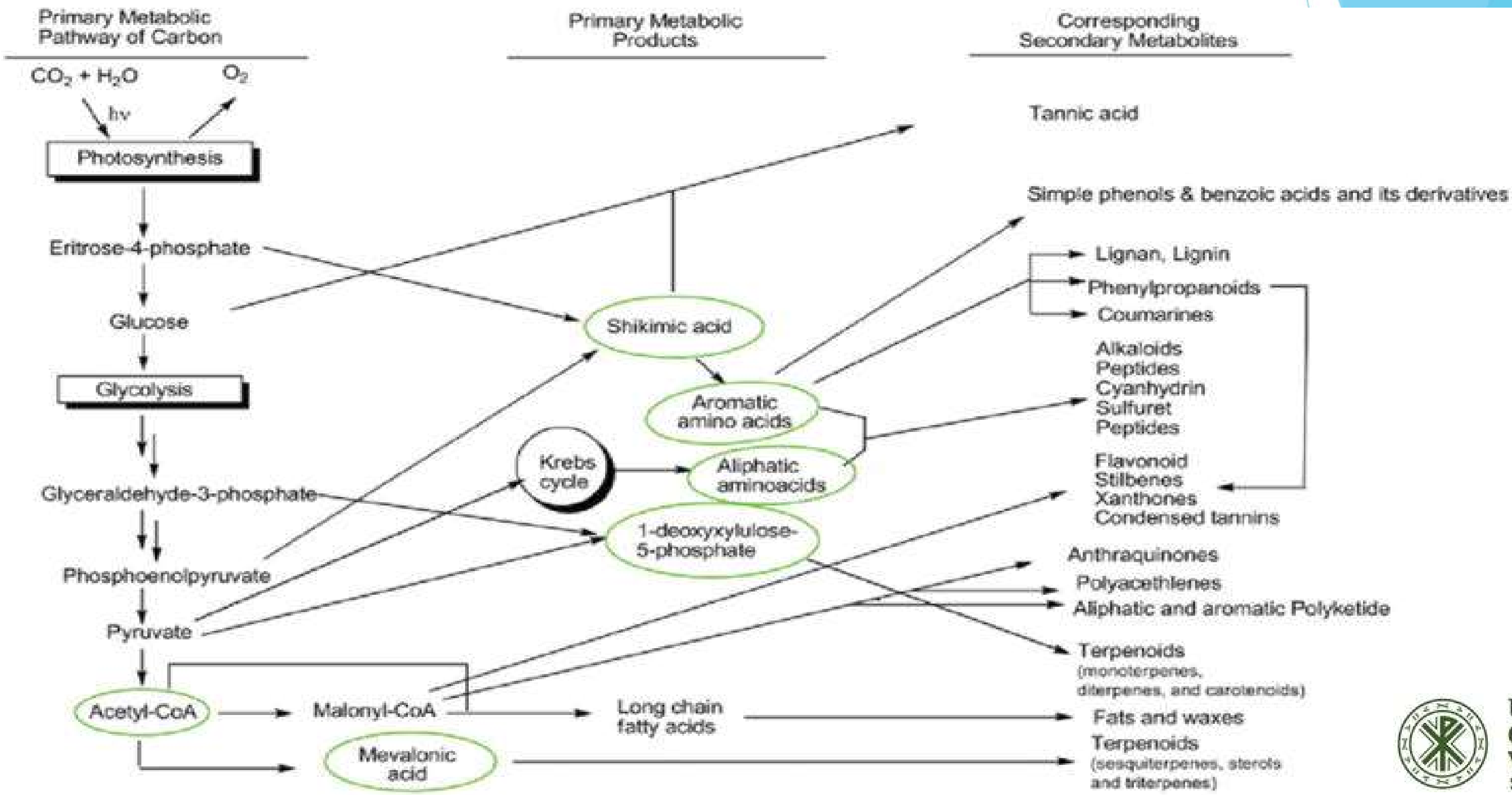
Discovering the chemodiversity



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Discovering the chemodiversity

PRODUCTION OF SECONDARY METABOLITES:

In a efficient metabolic regulation, the intermediate and terminal products of the primary pathways do not accumulate, specially if none specific genetic library to make secondary metabolites is present.

But some organisms have specific genes to produce secondary metabolites as well.

In these organisms, some specific steps of the primary metabolism lack regulation, resulting in the over-synthesis of terminal and intermediates products of the primary pathways.

Under metabolic stress, such accumulated reserves of intermediates products can induce subsidiary pathways to build secondary metabolites.

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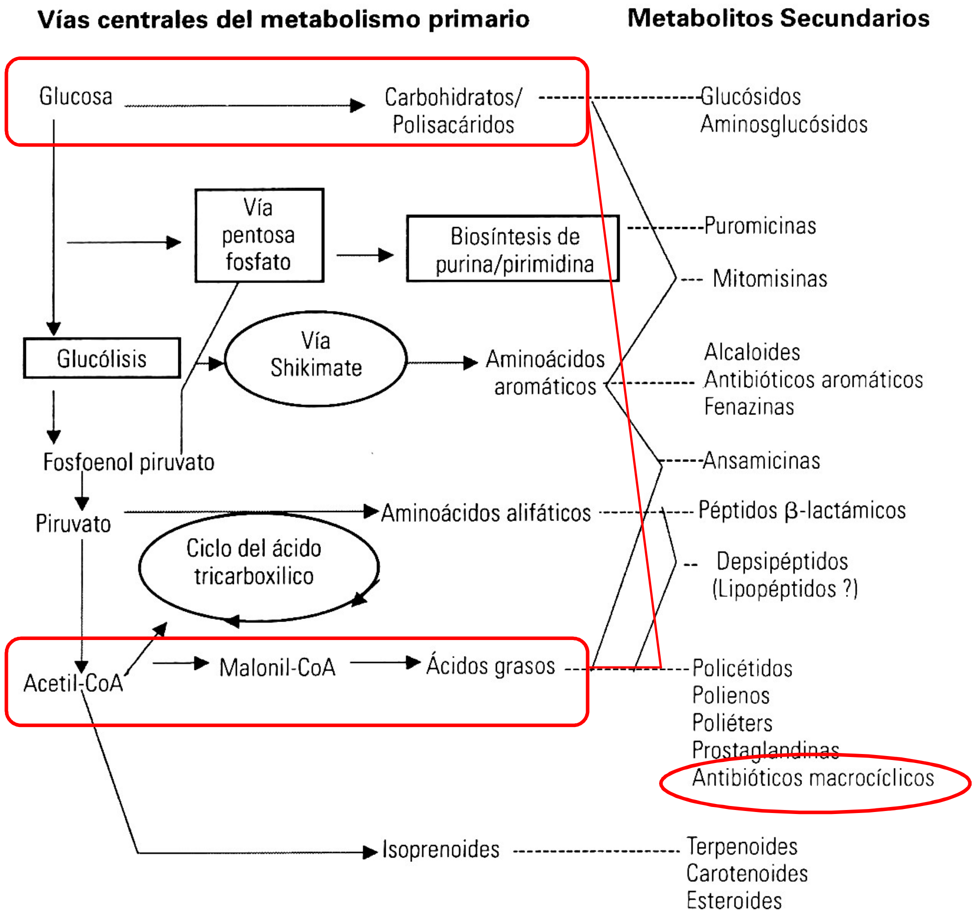
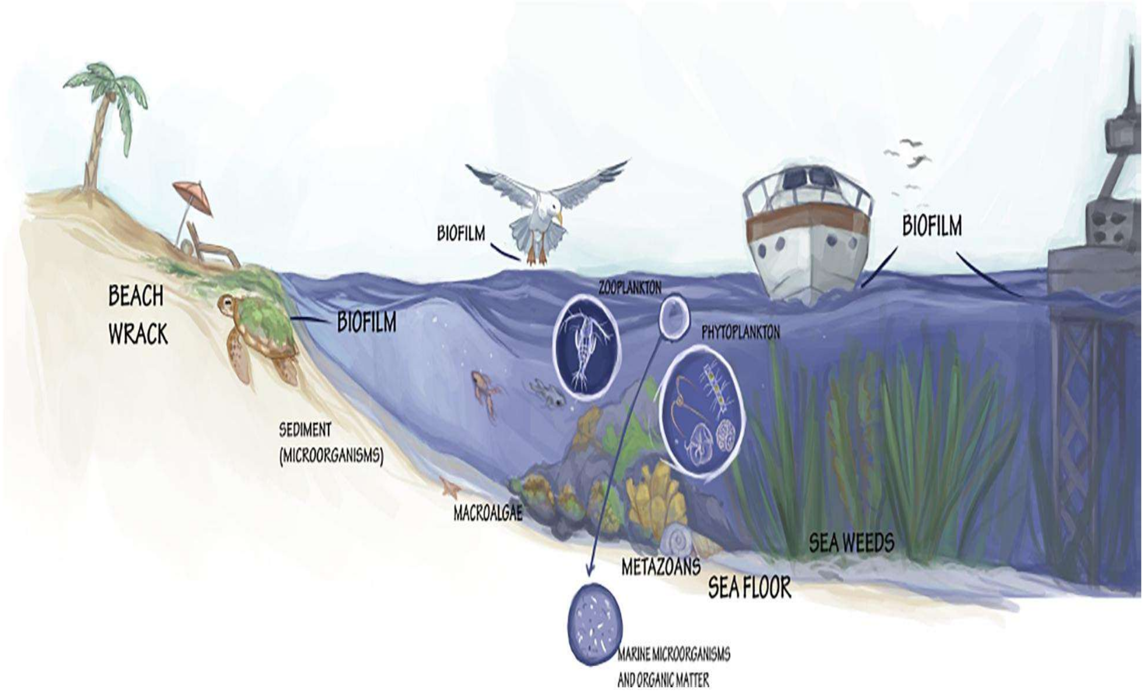
Discovering the chemodiversity



Example: the combination of the fatty acid biosynthetic network with monosaccharide biosynthetic pathways has resulted in the formation of macrolide antibiotics.

Discovery: where do we look for?

- Where competition, adaptation is required, ➔ anywhere



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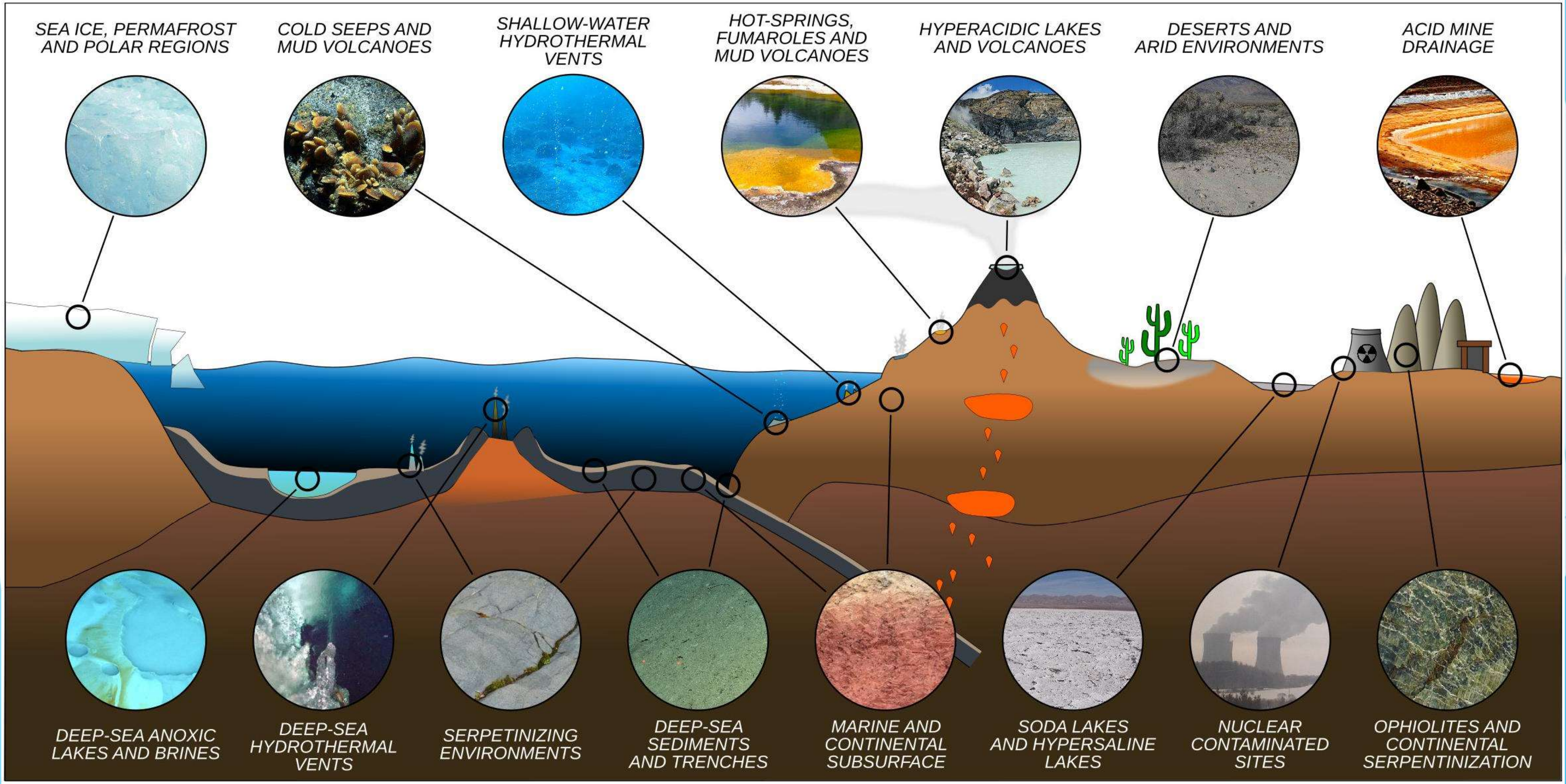
Discovery: where do we look for?

- Extreme habitats are very interesting, adaptation requirements are very high



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Discovery: where do we look for?

- Saltworks

Discovery: where do we look for?

- Hiperacid lakes

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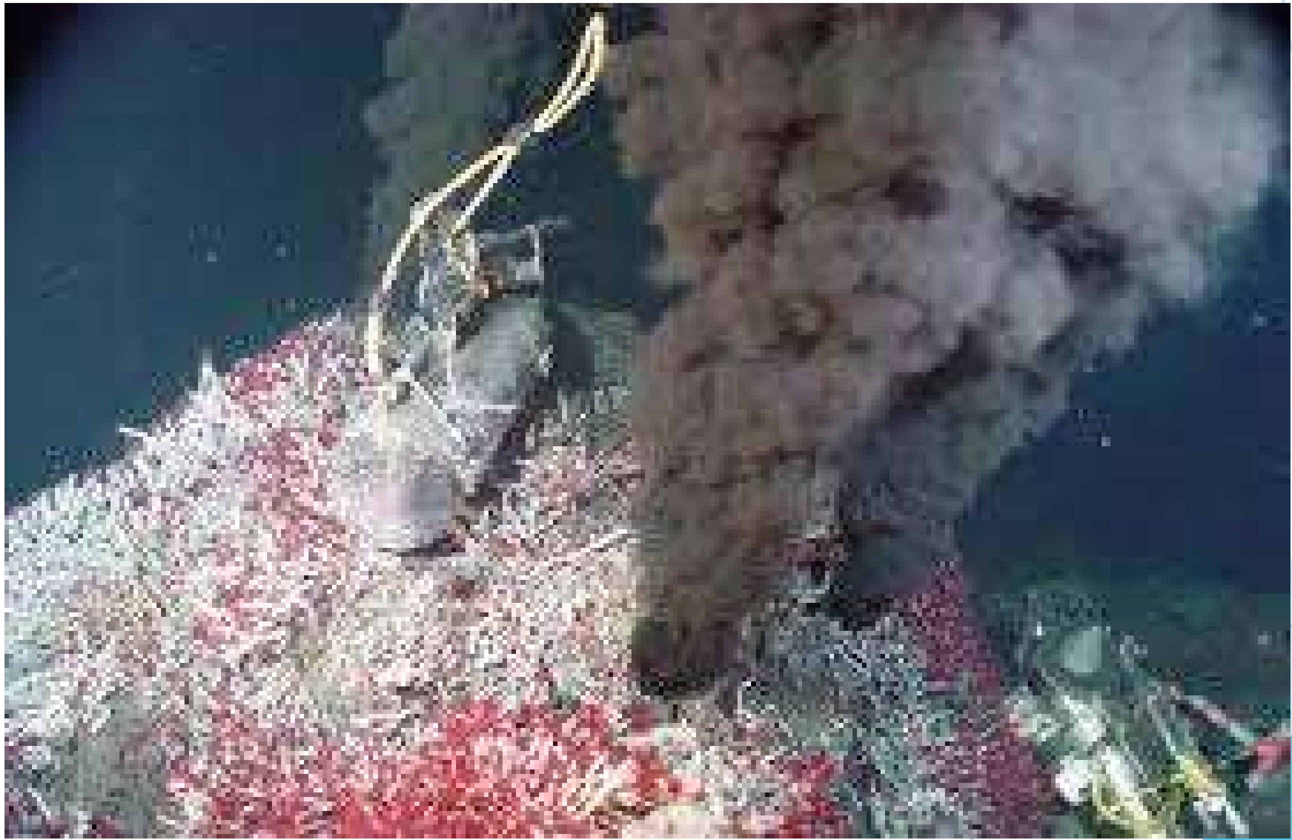
Discovery: where do we look for?

- Hydrothermal vents



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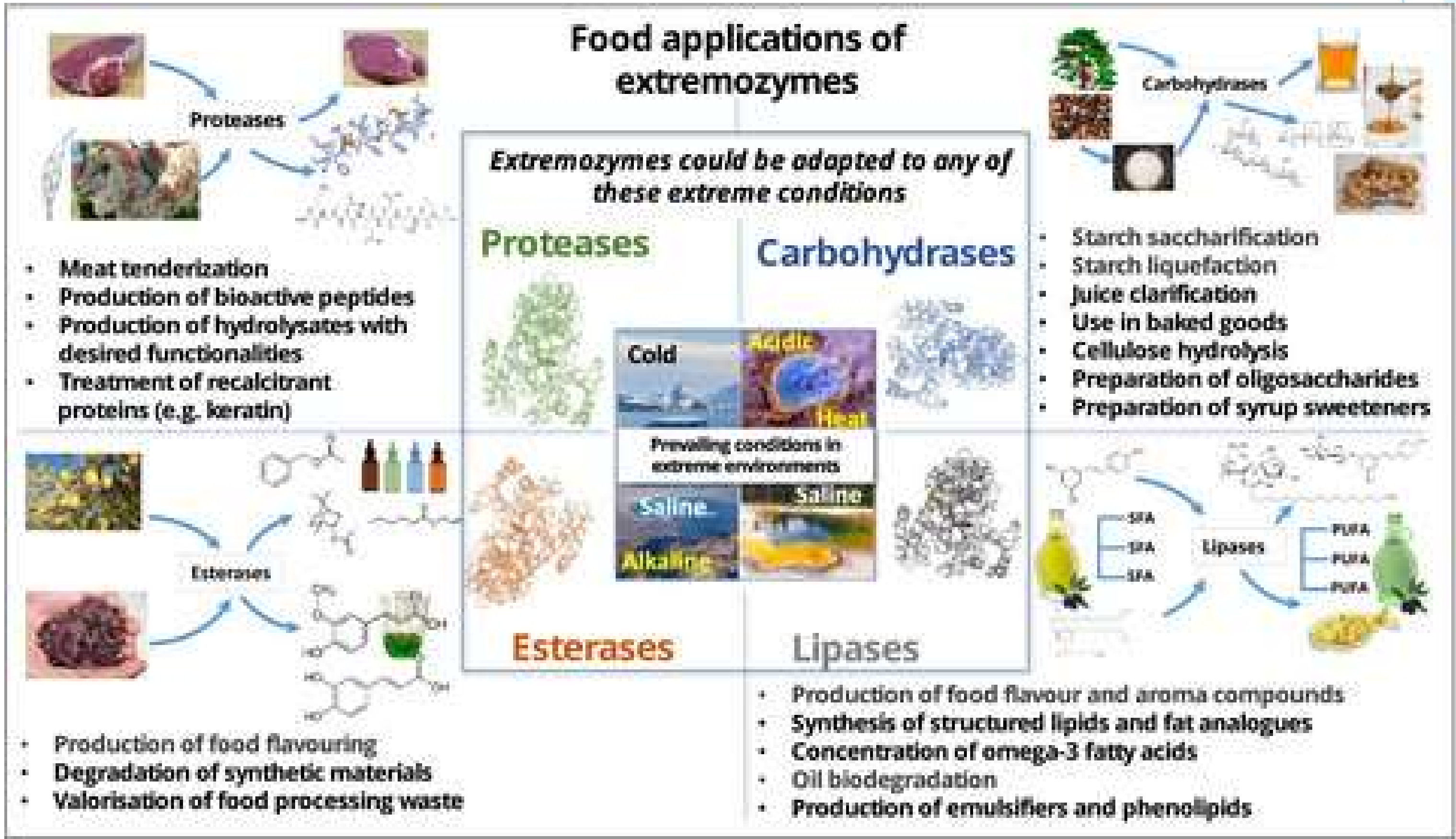
Discovery: where do we look for?

- What have we found in extreme habitats?



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Discovery: how do we look for?

- Prospecting:

Mission to explore genomic diversity of Indian Ocean

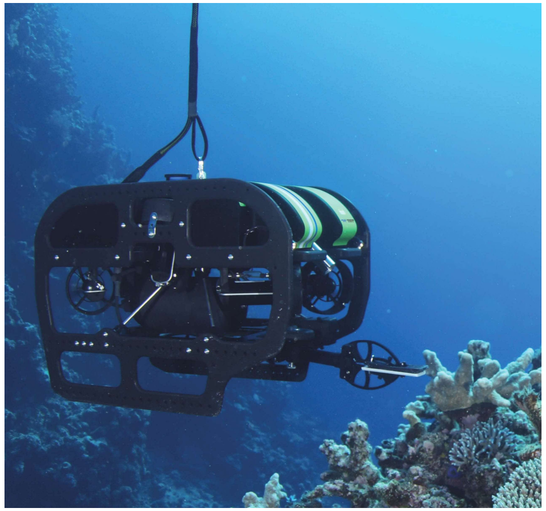
Vanita Srivastava

doi:10.1038/nindia.2021.37 Published online 14 March 2021

The scientists with the research vessel.

Discovery: how do we look for?

- Prospecting:



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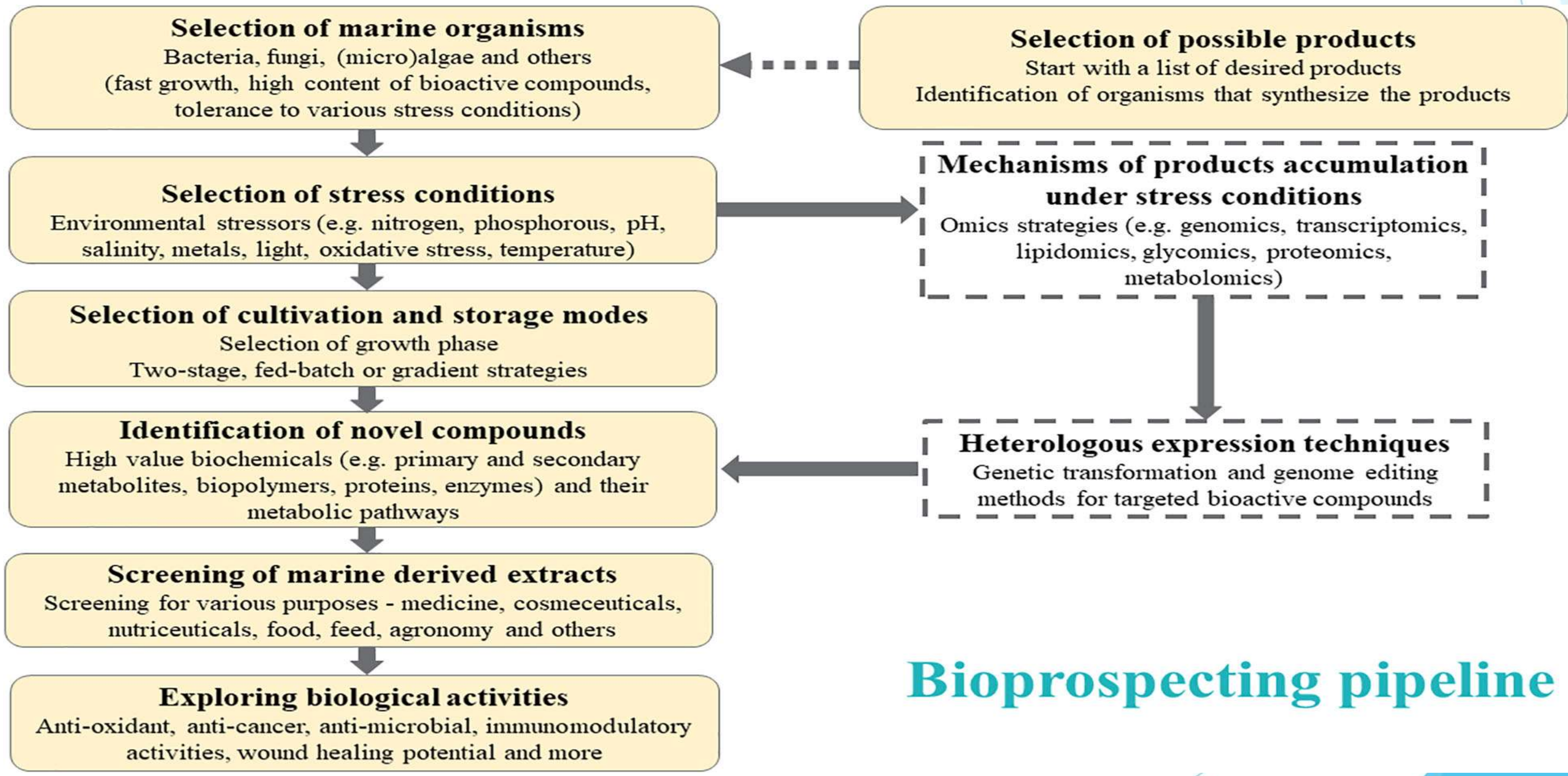
Discovery: how do we look for?

- Bioprospecting pipeline:



Blue Biotechnology findings

Source	Use	Representative phyla (exemplary genera/species)	Challenges
Metazoans	Medicine, cosmetics	Tunicates - Chordata (*Ecteinascidia turbinata*), Mollusca (*Conus magus*), sponges - Porifera (*Mycale hentscheli*), Cnidaria (*Sinularia* sp., *Clavularia* sp., *Pseudopterogorgia* sp.)	Sourcing and supply sustainability
Macroalgae and seagrasses	Food, feed, medicine, cosmetics, nutraceuticals, biofertilizers/soils conditioners, biomaterials, bioremediation, energy	Rhodophyta (*Euchema denticulatum, Porphyra/Pyropia* spp.*, Gelidium sesquipedale, Pterocladiella capillacea, Furcellaria lumbricalis, Palmaria* spp.*, Gracilaria* spp.), Chlorophyta (*Ulva* spp.), Ochrophyta (*Laminaria hyperborea, Laminaria digitata, Ascophyllum nodosum, Saccharina japonica, Saccharina latissima, Sargassum, Undaria pinnatifida, Alaria* spp., *Fucus* spp.), seagrasses (*Zostera*, *Cymodocea*)	Sourcing and supply sustainability Yield optimization, large-scale processing and transport Disease management
Microalgae	Sustainable energy, cosmetics, food, feed, biofertilizers, bioremediation, medicine	Chlorophyta (*Chlorella, Haematococcus*, *Tetraselmis*), Cryptophyta, Myzozoa, Ochrophyta (*Nannochloropsis*), Haptophyta (*Isochrysis*), Bacillariophyta (*Phaeodactylum*)	Bioprospecting and yield optimization (1 – increase in biomass/volume ratio, 2 – increase yield of compound/extract production and 3 – Improve solar-to-biomass energy conversion)
Bacteria and Archaea	Medicine, cosmetics, biomaterials, bioremediation, biofertilizers	Actinobacteria (*Salinispora tropica*), Firmicutes (*Bacillus*), Cyanobacteria (*Arthrospira, Spirulina*), Proteobacteria (*Pseudoalteromonas, Alteromonas*), Euryarchaeota (*Pyrococcus*, *Thermococcus*)	Culturing for non-culturable species, yield optimization
Fungi	Bioremediation, medicine, cosmetics, food/feed, biofertilizers	*Ascomycota* (*Penicillium*, *Aspergillus*, *Fusarium*, *Cladosporium*)	Limited in-depth understanding, yield optimization
Thraustochytrids	Food/feed, sustainable energy production	Bigyra (*Aurantiochytrium* sp.), Heterokonta (*Schizochytrium* sp.)	Limited in-depth understanding, yield optimization
Viruses	Medicine, biocontrol	Mycoviruses, bacteriophages	Limited in-depth understanc optimization

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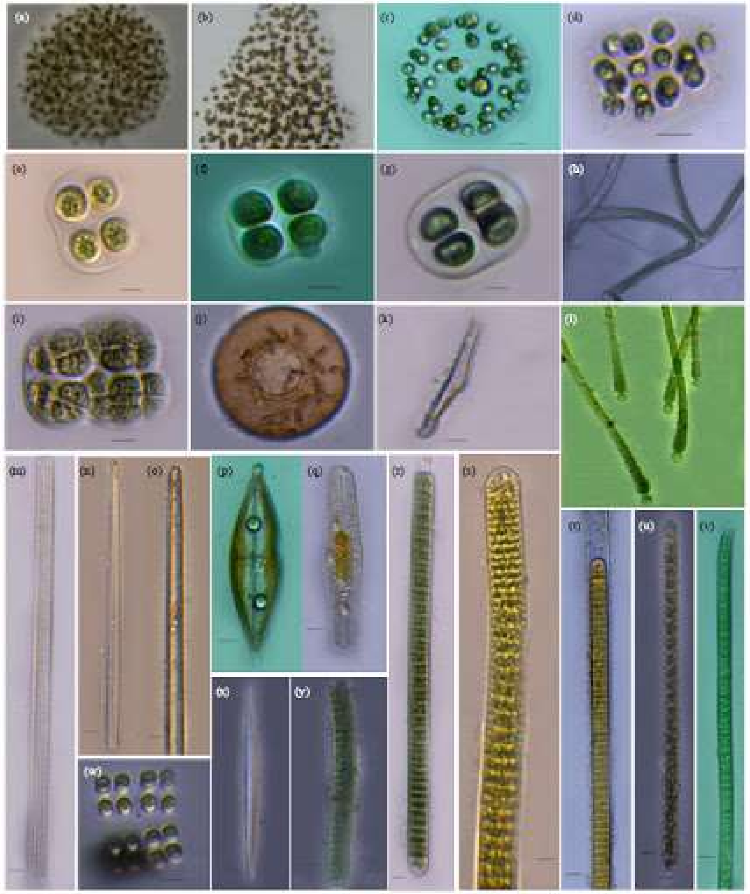
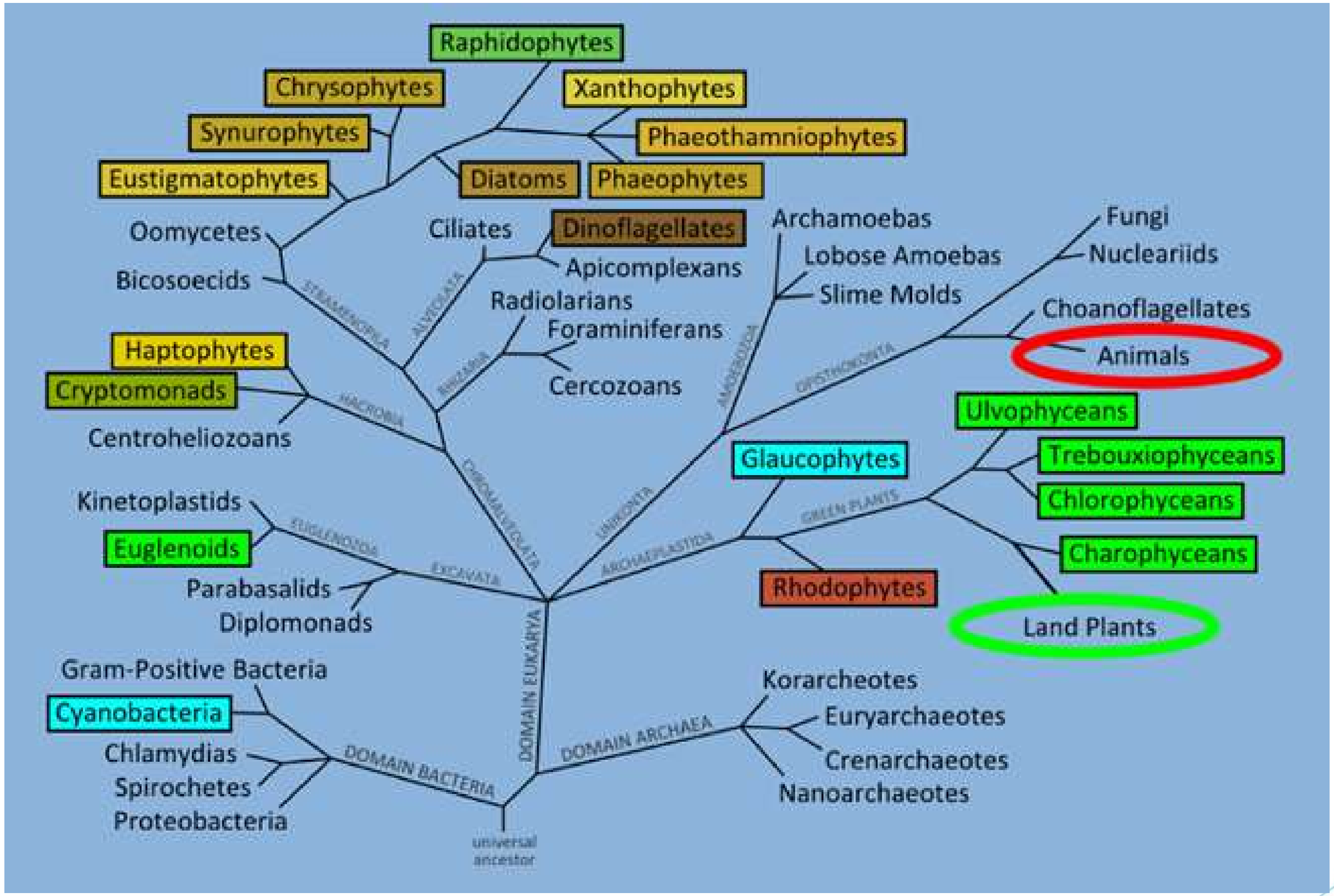
Microalgae diversity



- Microalgae are microscopic unicellular photosynthetic organisms, existing individually, or in chains or groups.(5-50 microns).
- Microalgae are an extremely diverse group of organisms, which include both prokaryotes and eukaryotes and covers 14 phyla with examples described from almost every possible habitat.
- An estimate of 200,000 – 800,000 species of microalgae is widely quoted, of which only about 35,000 are described.
- Microalgal habitats include freshwater, seawater, soils, and extreme environments.

Microalgae diversity



Phylogenetic tree highlighting the diversity and distribution of algae (boxed groups; colours indicate the diversity of pigmentation) across the domains of life (adapted from www.keweenawalgae.mtu.edu/).
For comparison animals and land plants are encircled in red and green, respectively.

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Microalgae diversity

- With simple life cycles and fast cellular growth rates, produces high concentrations in short times ➔ Red tides

Species/Strain selection processes

- Composition: lipids, carbohydrates, and proteins, but also pigments, antimicrobials, phytohormones,…
- Growth performance: high biomass yields
- Possibilities for industrial cultivation
- Stages for metabolites synthesis (one/two stages; stress conditions)
- Yields: biomass yield * hit concentration
- Availability: Culture Collections
- Strains improvement by selection or molecular engineering

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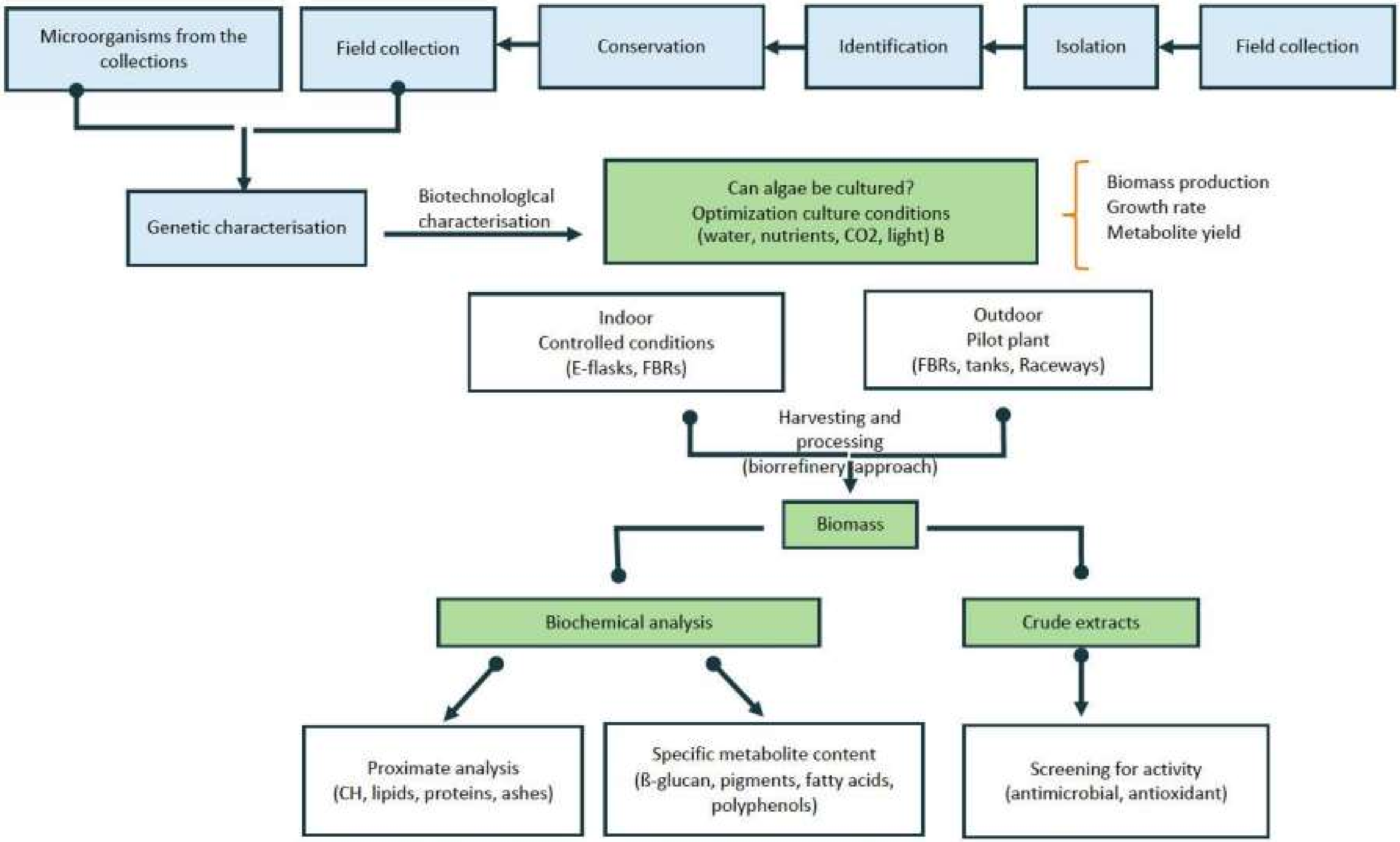
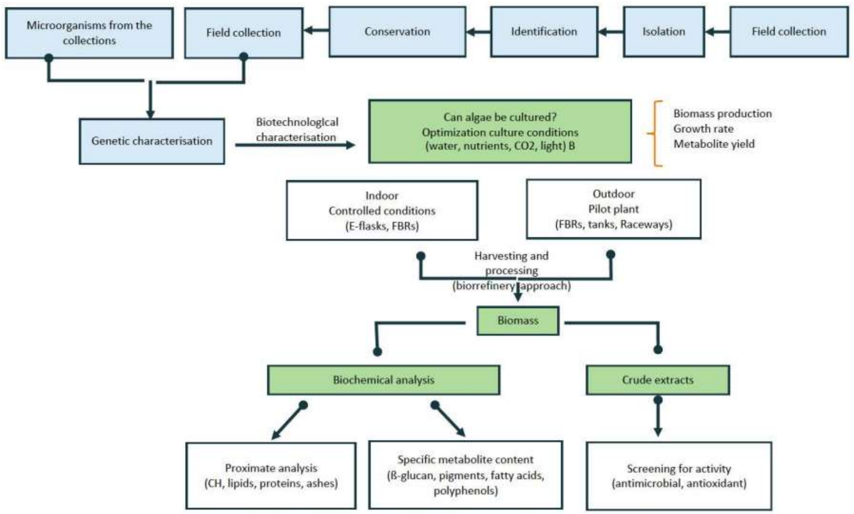
Species/Strain selection processes

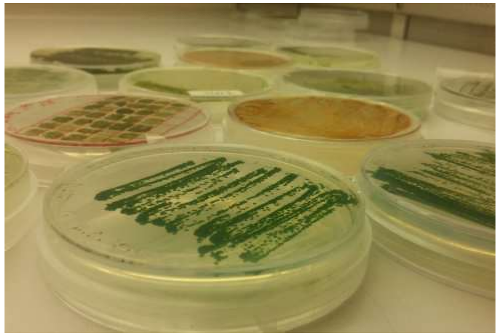


BELSPO/ULC and ULPGC – BEA Access Workflow example: Mass collection to extract metabolites

Species/Strain selection processes



BELSPO/ULC and ULPGC – BEA Access Workflow example: Mass collection to extract metabolites



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Upstream processes

Organic carbon and nutrient supply

- Bulk organic compounds
- Wastewater
- Processed materials
- Lignocellulose materials

Strain selection

Growth medium preparation

- Filtration
- Dilution
- Sterilization
- Hydrolisis

Light vs Dark

CO_2 vs O_2

Microalgae cultivation

Scaling up

- Culture scale:
  - Laboratory
  - Pilot
  - Commercial
- Growth modes
  - Photoautotrophic
  - Heterotrophic
  - Mixotrophic
- Bioreactor type:
  - Open systems
  - Photobioreactor
  - Fermenters
- Operation mode:
  - Batch
  - Fed-batch
  - Continuous
  - Semi-continuous

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Culture parameters

- Fundamentals:
  - Light gets to algae – light will not penetrate more than 5cm in a dense culture
  - Gas exchange (CO_2 in O_2 out); gas efficiency
  - Heat exchange – not too hot not too cold
  - Good mixing – shear forces, pH
  - Containment: good guys stay in bad guys stay out
  - Cost (CAPEX and OPEX)
  - Efficiency of land use; water; labour costs
  - Materials (longevity, sustainability; suitability)
  - Ease of cleaning
  - Ease of harvesting

Culture parameters: light

- natural or artificial: fluorescent tubes or LED technology
- Light parameters:
  - Quality of light: radiation spectrum (white, red, blue)
    - Production and accumulation of metabolites of commercial interest has been shown to be affected by white light radiation
  - Quantity of light: intensity (lux = w / cm2)
  - Photoperiod: 24h light, alternative Light/Darkness, flasing light

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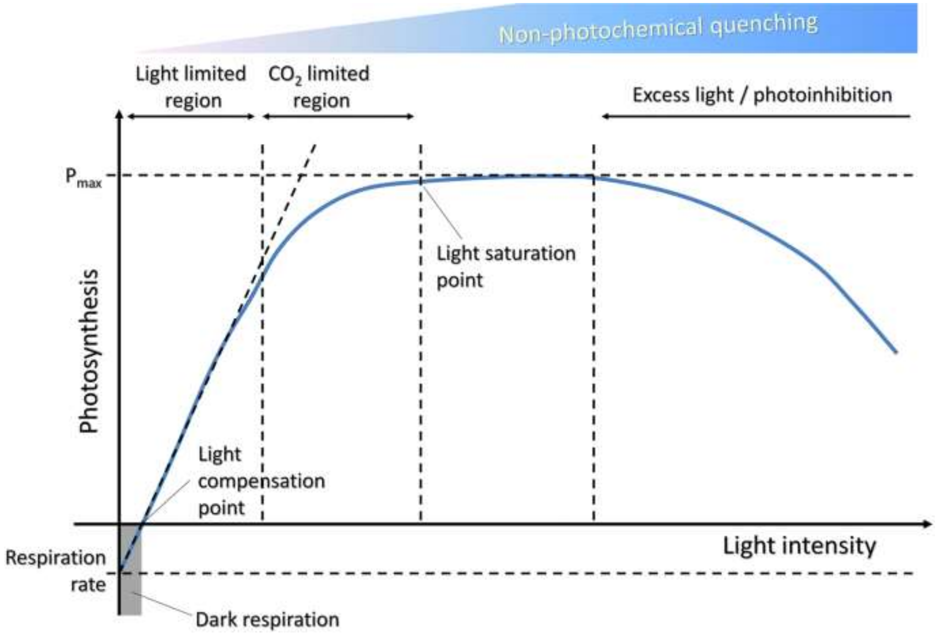
Culture parameters: light intensity



The specific growth rate of the microalgae depends on the intensity of the light

This growth pattern in relation to light intensity is observed in most microalgae species. However, the intensity and regimen of illumination vary with the genus of microalgae.

Culture parameters: CO2 / O2

Cuadro 1. Concentración de CO_2 tolerable para diversas especies de microalgas (Ono y Cuello, 2003)

Especie	Tolerancia máxima de concentración de CO_2
Cyanidium caldarium	100%
Scenedesmus sp.	80%
Chlorococcum littorale	60%
Synechococcus elongatus	60%
Euglena gracilis	45%
Chlorella sp.	40%
Eudorina sp.	20%
Dunaliella tertiolecta	15%
Nannochloris sp.	15%
Chlamydomonas sp.	15%
Tetraselmis sp.	14%

CO2 is major carbon source used in photoautotrophic microalgae cultures.

O2 is a photosynthesis by product, should be removed to avoid photosynthesis inhibition.

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Culture parameters: Temperature

Temperature is one of the most important environmental factors that affect the growth and development of living organisms.Therefore, it is required to know an optimal value for a maximum growth rate.

Comparatively with CO2 or light as limiting factors for photosyntesis, the influence of temperature is negligible. But, changes in temperature can also cause alterations in many of the metabolic pathways, including carotenoid biosynthesis. Adaptation is requested, and unexpected temperature changes can affect to growth performance.

Photosynthetic systems always generate heat because of the inefficiency of photosynthesis in converting light energy to chemical energy. (The theoretical conversion of red light to chemical energy is 31% and the remaining 69% is lost as heat). Therefore, the amount of cooling in a culture system will depend on the intensity of the light and the cell concentration, however, reactor cooling is only used in closed systems. Open systems use big volumes to stabilizes temperature inertia.

Temperature is also important for the dissociation of carbon molecules, making it available for photosynthesis. Temperature influences respiration and photorespiration more markedly than photosynthesis.

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Culture parameters: Temperature

Generally speaking, optimum temperature for the cultivation of microalgae is 20-24 ° C, however, these can vary depending on the culture medium, the species and the strain used.

Microalgae cultures commonly tolerate temperatures between 16 and 27 ° C.

Temperature <16 ° C decrease growth
Temperature > 35 ° C turns out to be lethal for a great number of species

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Culture parameters: pH

The pH range for most microalgae cultures is between 7 and 9, with an optimal range of 8.2 to 8.7 (although some authors claim that the optimum is at neutral pH 7.5).

An optimal pH in the culture is generally achieved through aeration with air enriched with CO_2.

As CO_2 is removed during photosynthesis pH increases; at alkaline pH levels photosynthesis is inhibited.

High cell density cultures, request of addition of carbon dioxide to prevent an increase in pH.

Buffer solutions are generally used to adjust and maintain the pH of the medium. The pH increases as the age of the crop is older, this is due to the accumulation of minerals and the oxidation of nutrients.

Some species (eg.: Arthrospira sp.) prefer high range of pH (9) to better growth performance, this avoid competition in open ponds.

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Culture parameters: Salinity

- Marine species have a fairly wide tolerance to salinity, between 12 and 40 ‰, with an optimum around 20 ‰
- Microalgae from hypersaline media (*Dunaliella, Asteromonas*) must be cultured in seawater or concentrated brines
- Tolerance depends on the ions present (osmolality)
- Salinity influences cell composition

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Culture parameters: mixing

Uniform dispersion of the microalgae in the culture medium, eliminating concentration gradients of light, nutrients (including CO_2) and temperature is reached by providing an efficient mixing system.

Excess mechanical agitation causes turbulence, which can cause permanent damage to the cell structure, affecting growth and metabolite production. On the contrary, insufficient agitation will cause sedimentation and cell death.

Culture parameters: mixing

- Few quantitative studies related to hydrodynamic stress have been carried out in microalgae cultures in photobioreactors.
- It is known that the increase in the growth rate of some species of microalgae when turbulence increases is due to the improvement of the supply of light and CO_2. However, at higher levels of turbulence, growth is drastically slowed, simultaneously increasing the surface velocity of the gas causing possible cell damage.
- Gas mixing systems or bubble column systems cause less cell damage than mechanical stirring systems. However, although these systems appear to cause less cellular damage, they are not exempt from causing cellular damage to a lesser extent.
- Finally, it is also essential to control the possible "mutual shadowing", produced by the continuous cell movement to and from the light and dark areas (essential to guarantee high biomass productivity).

Culture parameters: Inorganic nutrients

- Main inorganic nutrients are: N, P, addicionally for freshwater species: S, Cl, K, Mg, Ca, Na.

- Silicates are requested for diatoms cultures.

- Trace elements:
  - salts with metal ions such as Fe, Cu, Zn, Co, Mn, Mo
  - Na2EDTA: chelating agent

- Vitamins: tiamine (B1), biotine (B8) y cianocobalamine (B12)

- There are several formulated culture media available (research, commercial) to cover the requirements for each group of microalgae.

- In addition, according to the objective of the experiment/production, the composition of the culture medium will be defined. For example, if the goal is high biomass productivity (g / L), high concentrations of nitrates and phosphates will be required. Instead, to induce the production of metabolites and compounds of commercial interest, the nitrate concentration is manipulated to simulate a stressful environment.
- An excess of Fe can stimulate the accumulation of astaxanthin in *Haematococcus pluvialis*.

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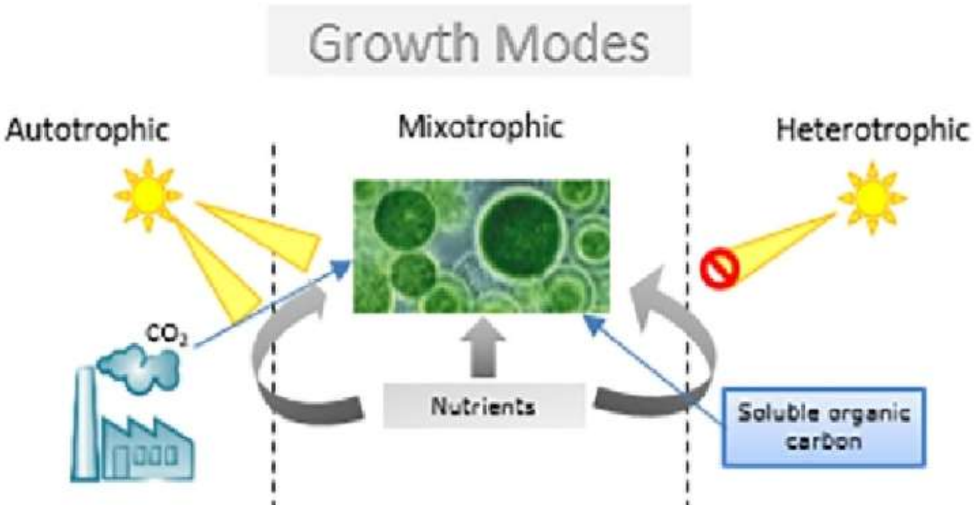
Culture nutritional modes

Growth mode	Energy source	Carbon source	Light availability requirements	Metabolism variability
Photo-autotrophic	Light	Inorganic	Obligatory	No switch between sources
Heterotrophic	Organic	Organic	No requirements	Switch between sources
Photoheterotrophic	Light	Organic	Obligatory	Switch between sources
Mixotrophic	Light and organic	Inorganic and organic	No obligatory	Simultaneous utilization



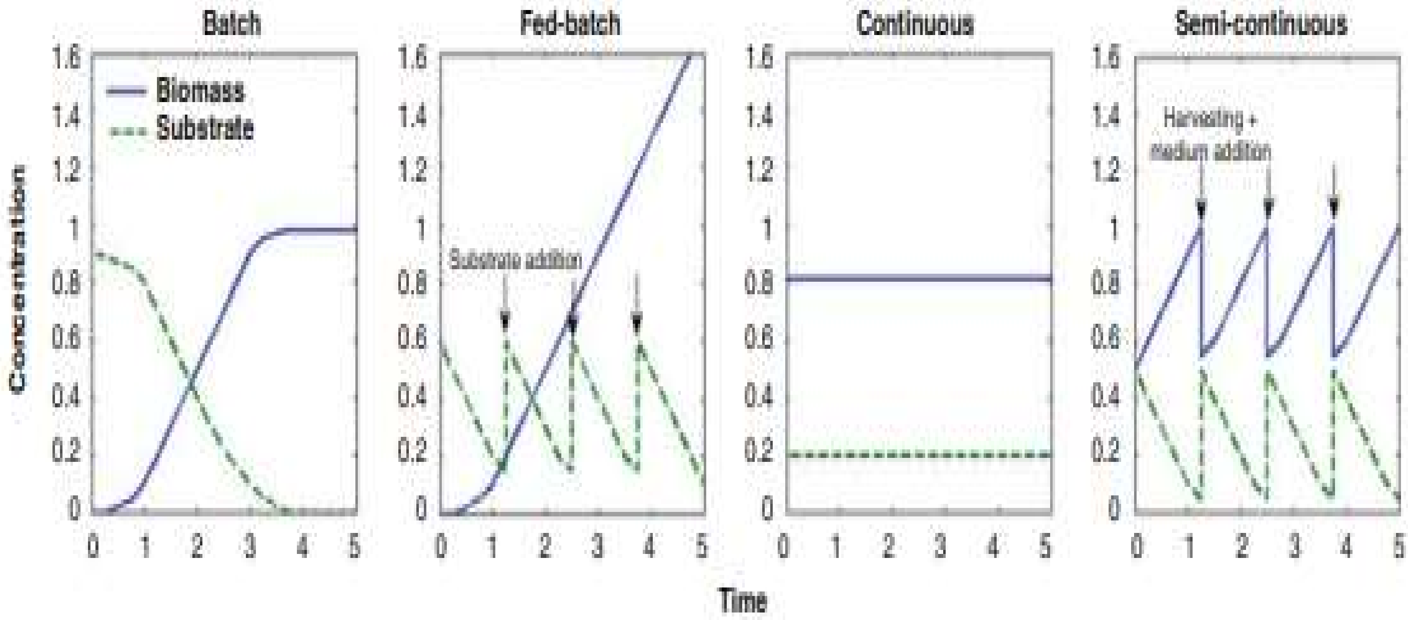
Culture nutritional modes

Table 2 Challenges and opportunities for the heterotrophic/mixotrophic cultivation of microalgae

Limitation	Opportunities
Carbon sources costs	Investigate new sources of cheap organic carbon, such as wastewaters, lignocellulosic material, and industrial processes waste
	Bio-prospection of strains able to assimilate cheap carbon sources
	Metabolic engineering of strains able to assimilate cheap carbon sources
	Improve methods for breakdown of lignocellulose material
Competition by fast-growing bacteria	Development of mixotrophic cultivation strategies
	Establishing cultures of microalgae able to thrive under bacteria-adverse environmental conditions
	Bio-prospection of fast-growing strains
	Metabolic engineering of fast-growing strains
	Immobilization of microalgae in polymers
Bioreactor implementation and operation costs	Cheaper materials for bioreactor vessel
	Implement alternative mixing strategies powered by a renewable energy source (hydraulic or wind)
	Implement cheap sterilization strategies
	Establish non-axenic microalgae cultures, such as open ponds
	Increase productivity of the metabolites of interest by optimizing bioreactor's operation parameters
	Risk assessment studies and regulations of GMOs in large-scale facilities
Downstream processes costs (biomass harvesting and raw product transformation)	Enhance exo-polysaccharides production to promote biomass flocculation
	Develop immobilization technique for the algae in polymeric beads/sheets
	Promote spontaneous excretion of metabolite of interest
	Selection or design of strains that excrete products
	Avoid compound extraction and separation by directly transform the biomass to products by pyrolysis, anaerobic digestion, gasification.

- Nutritional mode affects:
  - Growth performance
  - Metabolite accumulation
  - Operation costs
  - Production management

Culture operational modes



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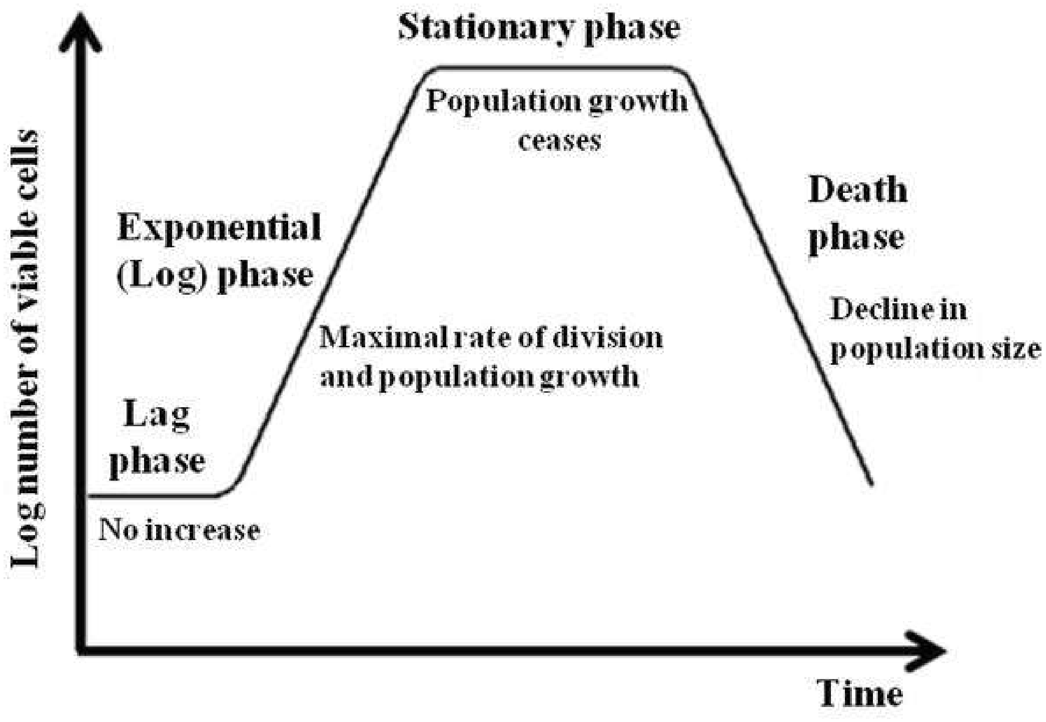
Culture dynamics:



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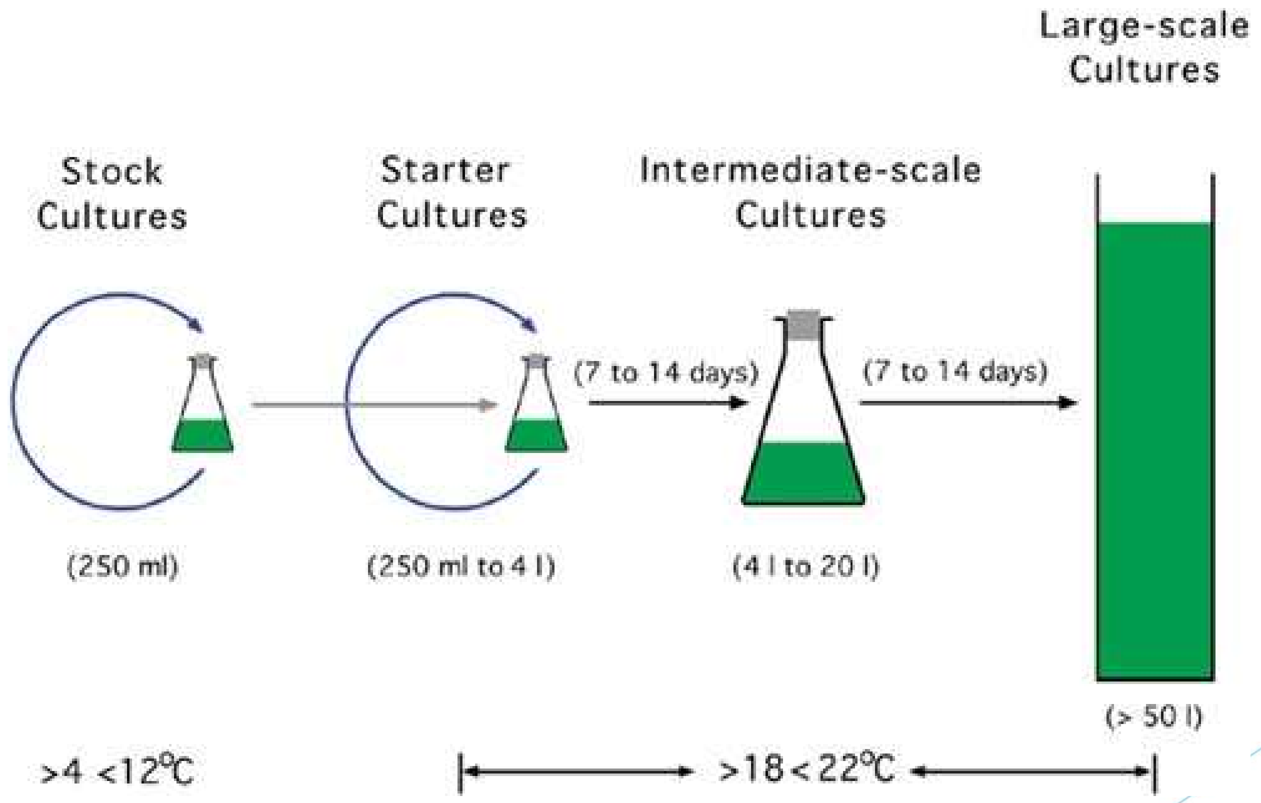
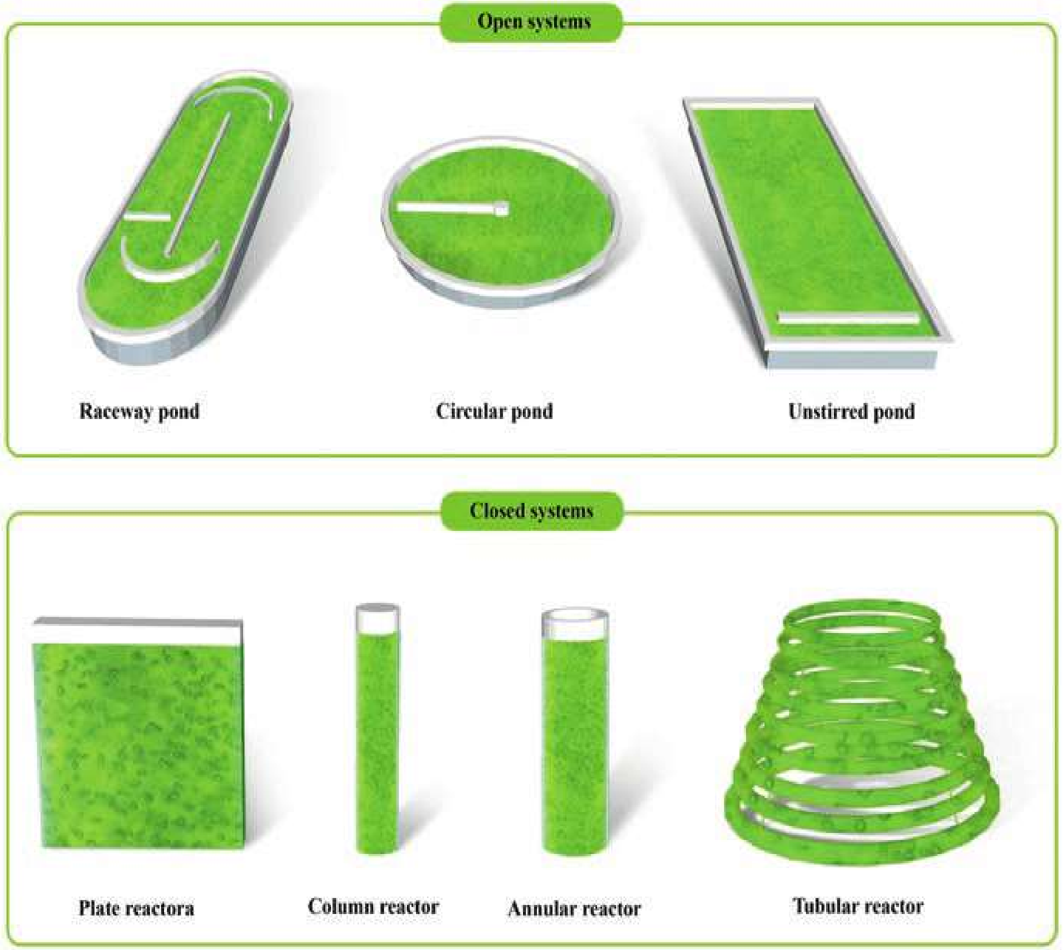
Culture dynamics: scaling-up



Culture systems: open/close; outdoor/indoor



Both systems can be:

- Outdoor
- Indoor

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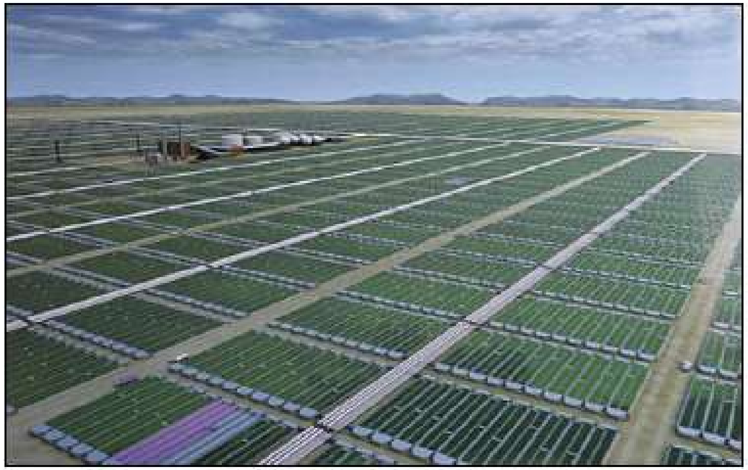
Culture systems: open systems

aquafeed.co

making-biodiesel-books.com



www.naturalcooling.com

ccresaquaponics.wordpress.com

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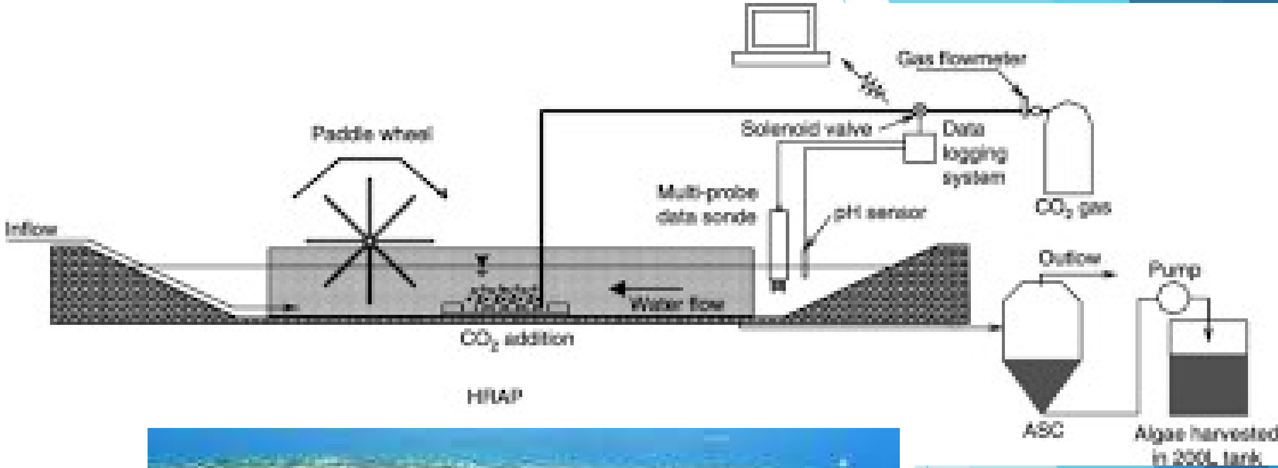
Culture systems: close systems

Culture systems: open systems → outdoor Ponds



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Culture systems: open systems → close Ponds

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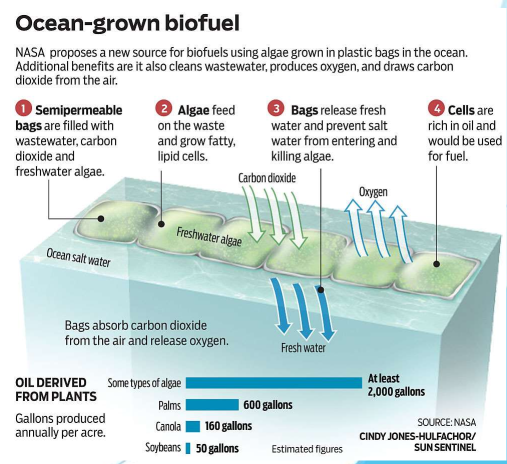
Culture systems: close systems ➔ bags



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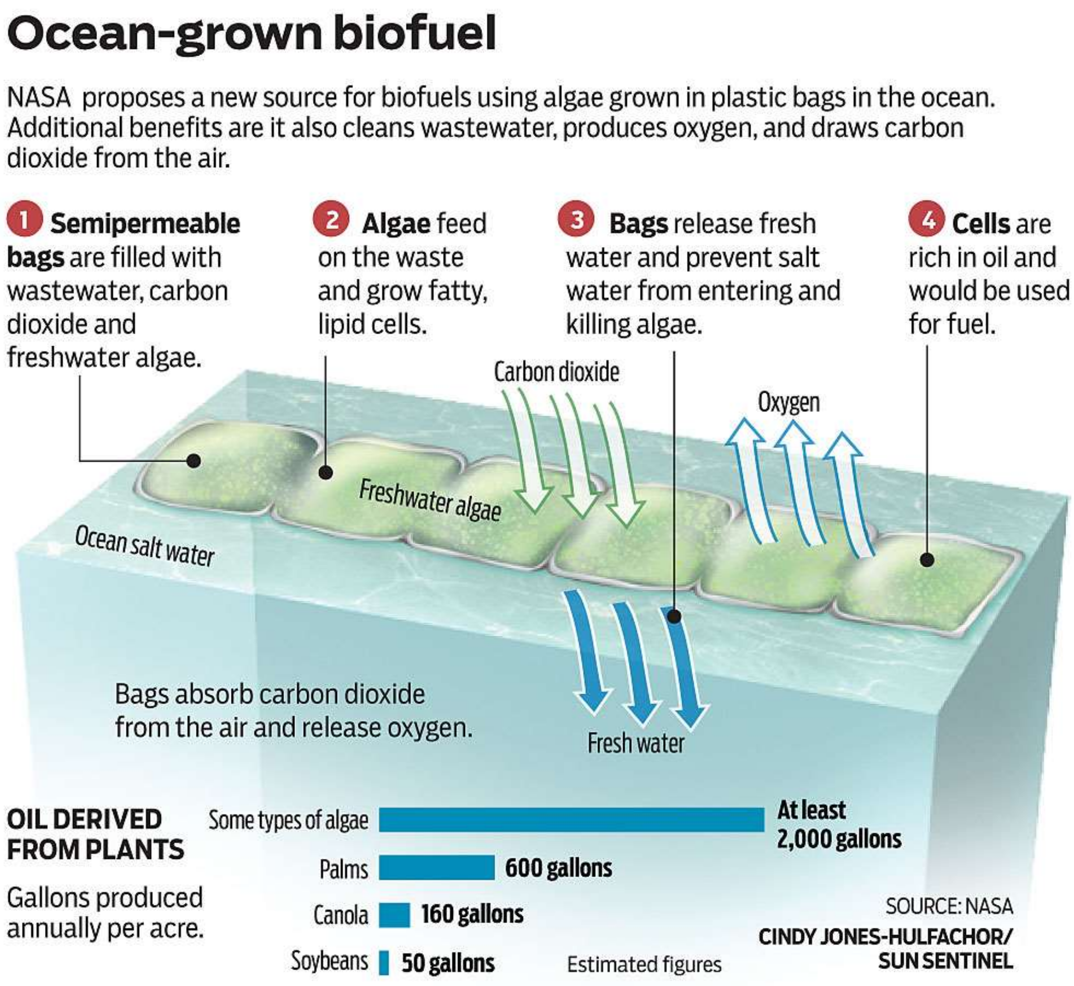
Culture systems: close systems ➔ bags



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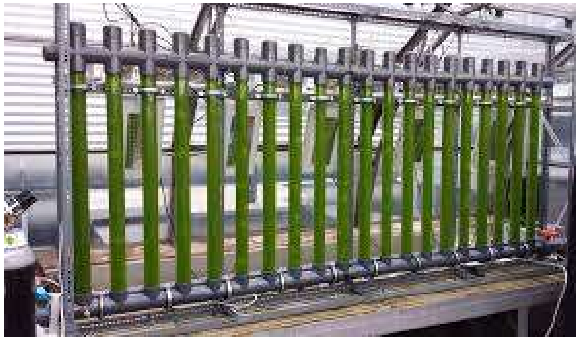
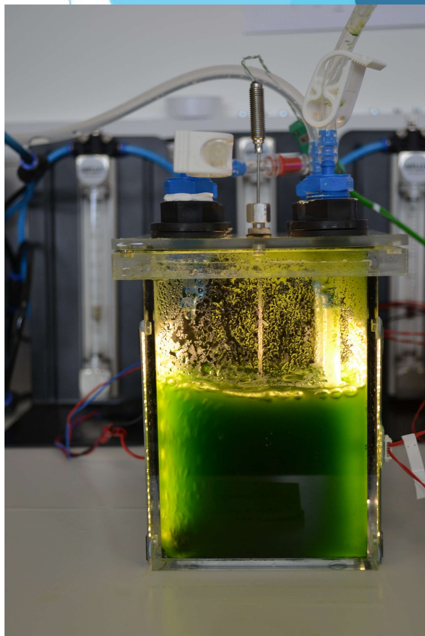
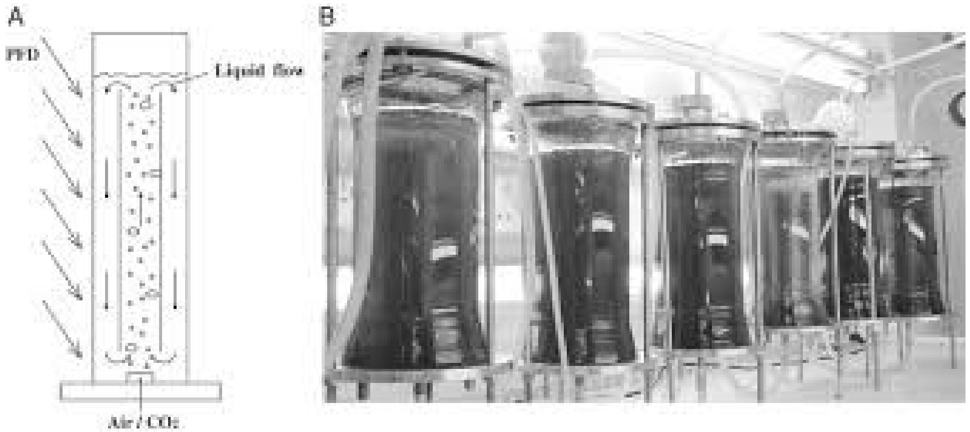
Culture systems: close systems ➔ column PBRs



Bubble and Air Lift driven PBRs

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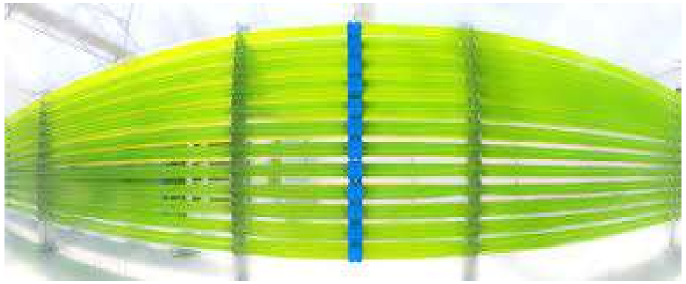
Culture systems: close systems → tubular PBRs



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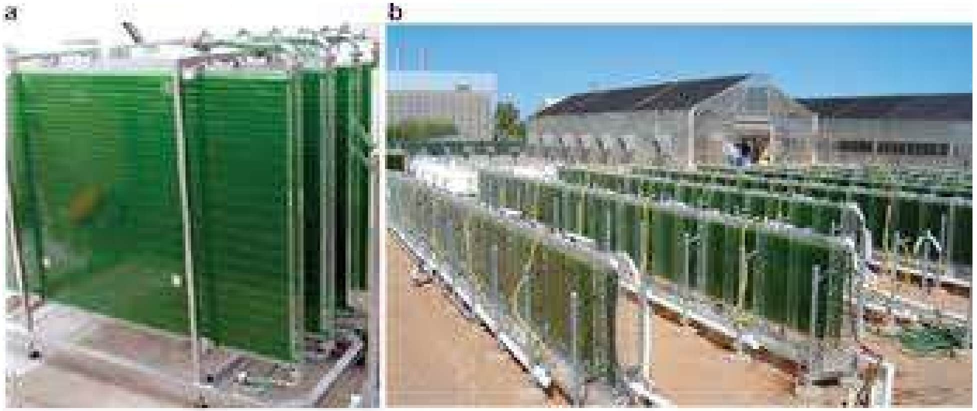
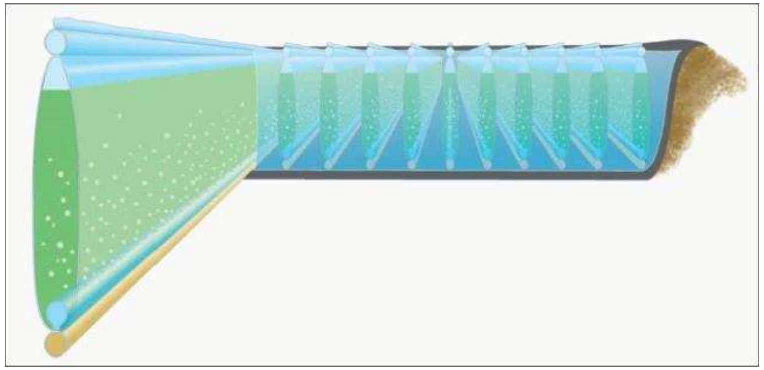
Culture systems: close systems ➔ tubular PBRs

Glass vs plastics

Culture systems: close systems ➔ flat panel PBRs





Maintenance of mass cultures

Objective: obtaining cost-effective productivity of biomass and compounds

Action: updating continuous information for assessment of culture performance

Tools: culture performace parameters:

- **On-line monitoring of photosynthetic activity**
  - Measuring disolved oxigen (DO):
    - Reliable and sensitive indicator of state of culture, in relation to growth and productivity.
    - Excess of DO may result in:
      - decrease of yield of cell mass and pigment content.
      - Promote photoinhibition and photo-oxidation => culture death
      - Ineplicable decrease or decline of DO => culture is stressed and may quick deteriorated.
    - In situ monitoring of chlorophyll fluorescence
      - Well complement DO measurements for rapid and accurate assessment of welfare of culture.

Maintenance of mass cultures

Tools: culture performace parameters:

- **Measurement of cell growth and culture productivity**
  - Net growth may be estimated quickly by measuring changes in the overal turbidity (Optical density) of the culture.
  - OD is a rough estimation of growth and should be followed routinely by cell count, dry weight or total organic carbon.

- **Night biomass lost**
  - If light/dark cycles, also should be temperature cycles that could affect to the culture.

- **Maintaining optimal cell density (OCD / OPD)**
  - Population density represents a major parameter in the production of photoautotrophic mass.
  - OCD = concentation in continuous cultures that results in the highest output rate of biomass and/or desired products.
  - Since culture is most stable when population density is optimal => continuous cultures should be mantained at that density.
  - OCD is determined empirically.

Maintenance of mass cultures

Tools: culture performace parameters:

- **Preventing nutritional deficiences**:
  - Routine test to check any posible deficiency of mineral nutrients.
  - Monitoring [N] could be a guideline for adding in proportional ammounts the entire nutrient formula.
  - Carbon and phosporous should be monitored and added separately.
  - Be aware that deplection could affect to minor nutrients outside nitrogen utilization => scheduled replacing of 50% of culture to secure nutritional sufficiency and nutrient balance.

- **Maintenance of monoalgal cultures and combating contamination**:
  - Foreing algal species, grazers and predators /amoebas, cilliates, rotifers, fungi
  - Buildup in microorganisms number could indicate that some nutrient has declined and the culture is under stress.

Microalgae Downstream process ➔ From biomass to markets

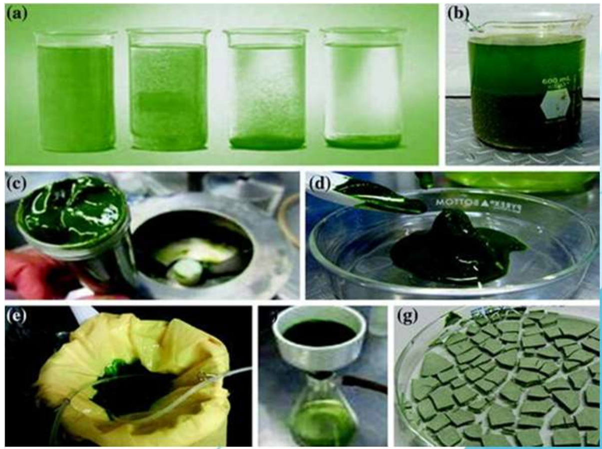
➔

Microalgae Downstream process ➔ From biomass to markets

Algae provide valuable nutrients to the human diet. They are rich in dietary fiber, such as beta-glucan (prebiotics), and some species have significant amounts of protein of higher quality than plant sources like beans and grains. Some algae produce plentiful polyunsaturated fatty acids such as omega-3 fatty acid. Algae also contain vitamins and minerals.

Additionally, studies show algae's potential to act as a prebiotic, anti-inflammatory, anti-cancer, neuroprotective, antidiabetic, anticoagulant, and immunomodulating agent.

Unfortunately, microalgae processing technology is highly underdeveloped, making it expensive, particularly regarding **harvesting and dewatering**. Significant research and technological improvements are necessary before microalgae can become a widely utilized protein source.



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Microalgae Downstream processes

Microalgae cultivation

Reused water

Electricity generation

Biomass harvesting → Extraction → Separation → Transformation → Purification

Biomass cake by-pass

Biomass harvesting:

- Flotation
- Flocculation
- Centrifugation
- Filtration
- Dewatering

Extraction:

- Solvent extraction
- Superficial fluid extraction
- Ultrasonic-assisted
- Enzymatic

Transformation:

- Fermentation
- Anaerobic digestion
- Transesterification
- Pyrolisis
- Gasification (Syngas)
- Combustion
- Hydrotermal liquefaction

Products:

- Biofuels
- Lipid based food
- Pigment based food
- Protein based food
- Protein based feed
- Sugars
- etc

Microalgae Downstream processes

Processing is becoming a major área of R&D in microalgal biotechnology => bottleneck to commercialization of microalgal products.

Supplying in situ produced whole algal cultures as feed to zooplancton (rotifer, artemia) or bivalve mollusks at aquaculture hatcheries is easy.

Major obstacle on industrial scale for production of value added products => **harvesting and dewatering steps**

Recovery biomass can be a significant problema because of:

- Small size of algal cells (2-30 microns)
- Low gravity settlling speed (< 10^{-5} m/s)
- Low biomass concentration in culture médium (< 5 gr DW/l)

Microalgae Downstream processes

Harvesting methods for microalgal cultures:

- Physical methods:
  - **Sedimentation**
    - tendency for particles in suspension to settle out of the fluid in which they are entrained and come to rest against a barrier.

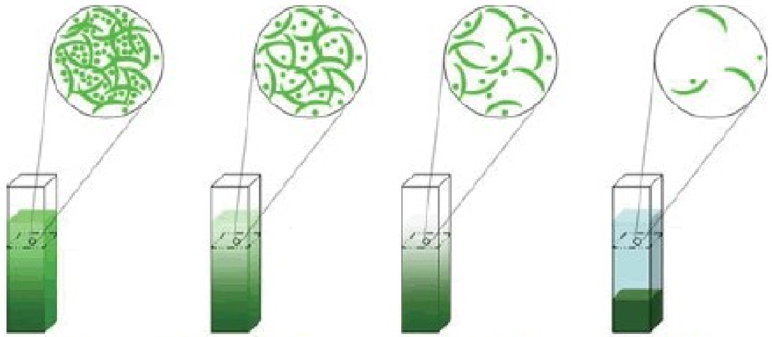

Figure 1: Sedimentation process of microalgae over time [19].

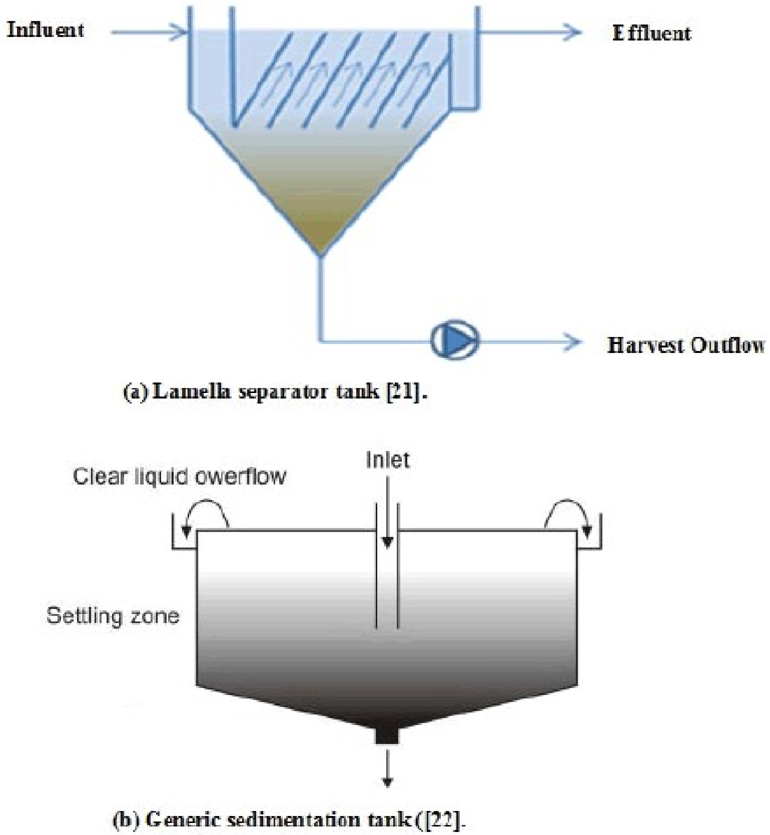

Figure 2: Sedimentation tanks.

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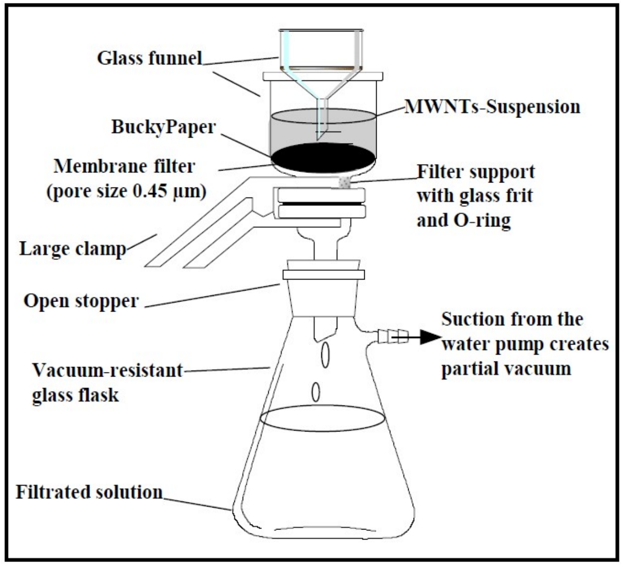
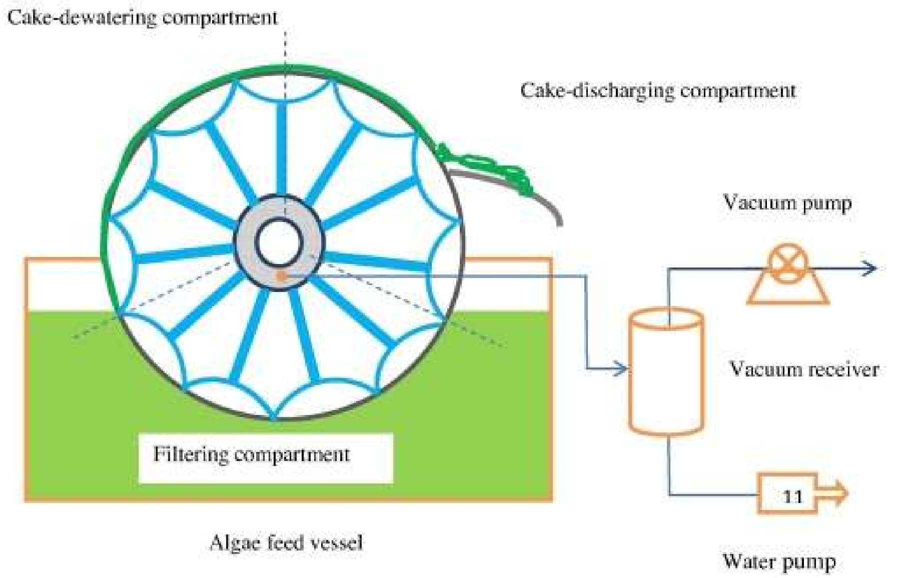
Microalgae Downstream processes

Harvesting methods for microalgal cultures:

- Physical methods:
  - **Filtration**
    - Vaccum filtration

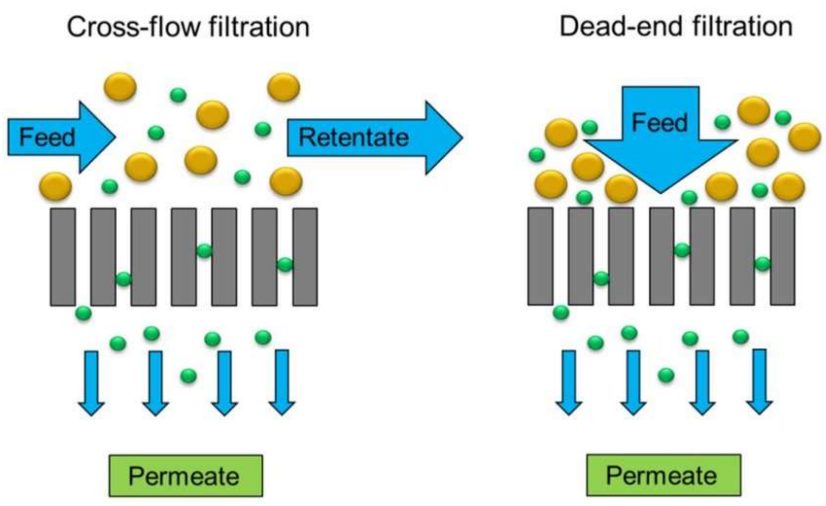




Microalgae Downstream processes

Harvesting methods for microalgal cultures:

- Physical methods:
  - **Filtration**
    - Pressure filtration (Dead-end filtration
    - Cross Flow filtration



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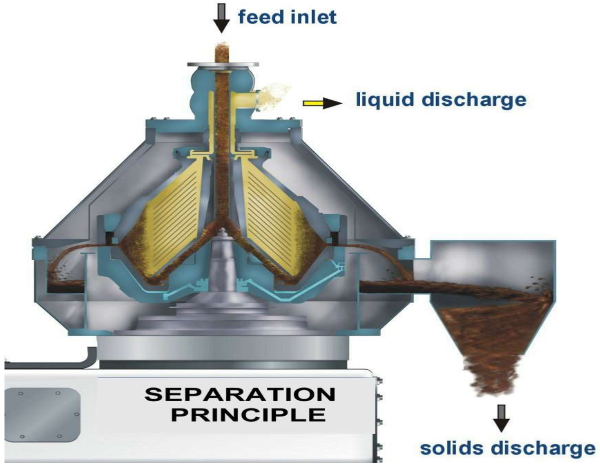
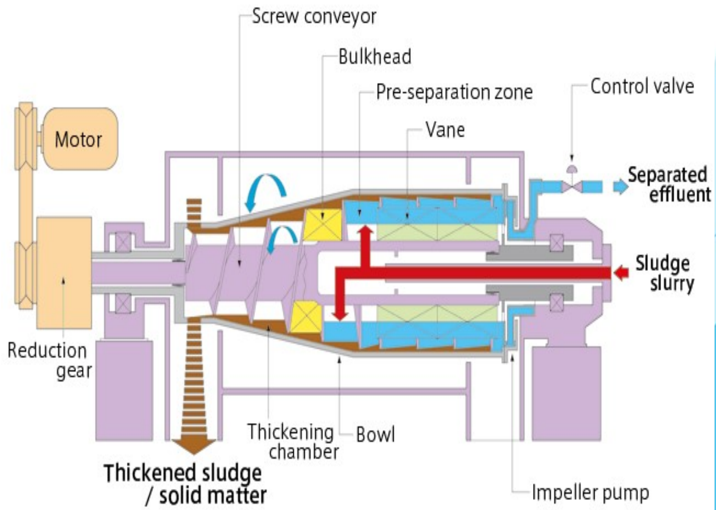
Microalgae Downstream processes

Harvesting methods for microalgal cultures:

- Physical methods:
  - **Centrifugation (> 10,000 rpm)**
    - Disc stacks centrifugue
    - Decanter centrifugue





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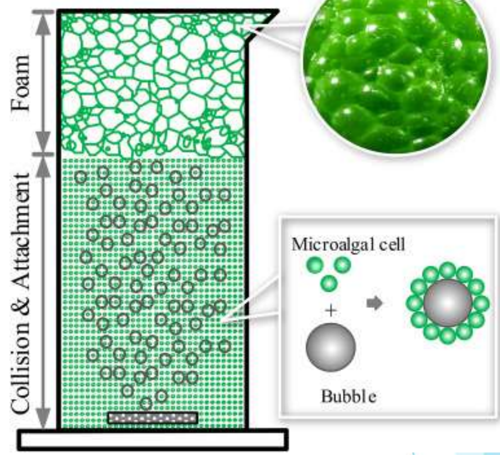
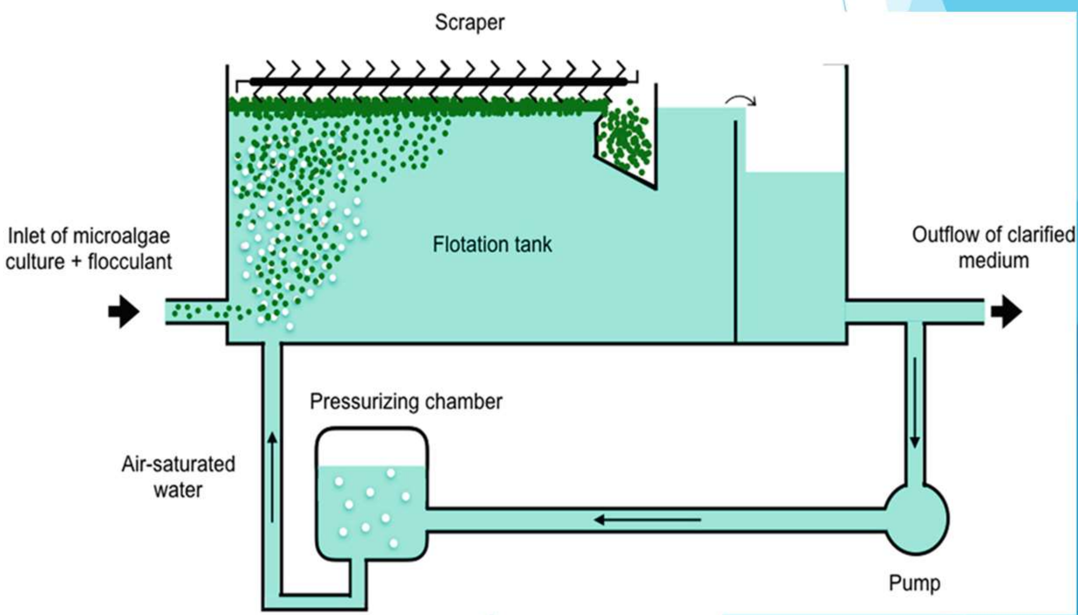
Microalgae Downstream processes

Harvesting methods for microalgal cultures:

- Physical methods:
  - **Flotation**
    - Dispersed air flotation
    - Dissolved air flotation (DAF)





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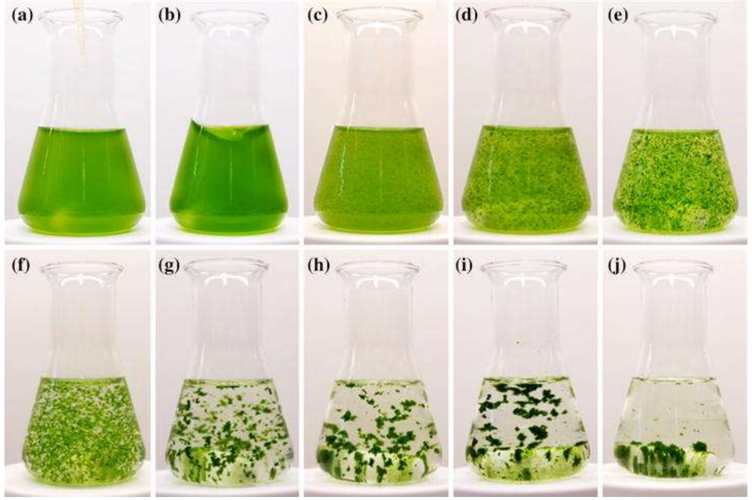
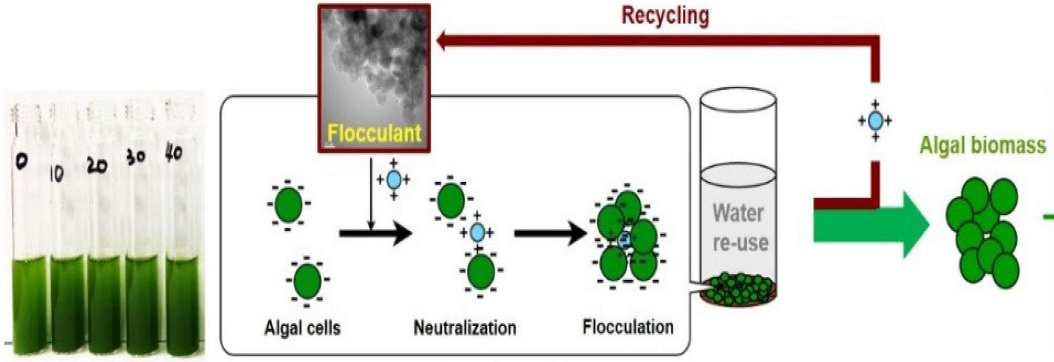
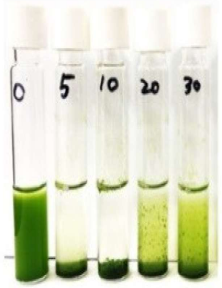
Microalgae Downstream processes

Harvesting methods for microalgal cultures:

- Chemical methods:
  - **Flocculation**
    - Inorganic flocculants
    - Organic flocculants.
    - Polimeric flocculants.







Flocculation of Scenedesmus acuminatus using flocculant PK55H. a Addition of flocculant, b flocculant distribution, c destabilization of the suspension, d, e floc formation, f–i floc growth and j settling of the flocs

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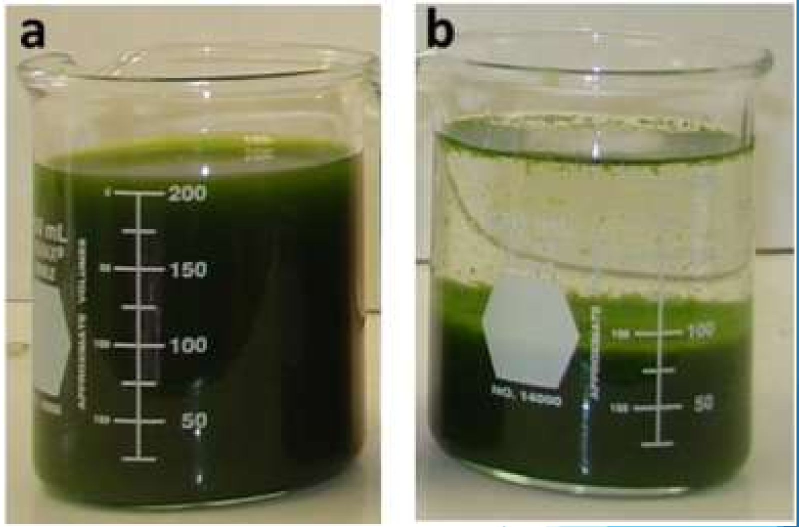
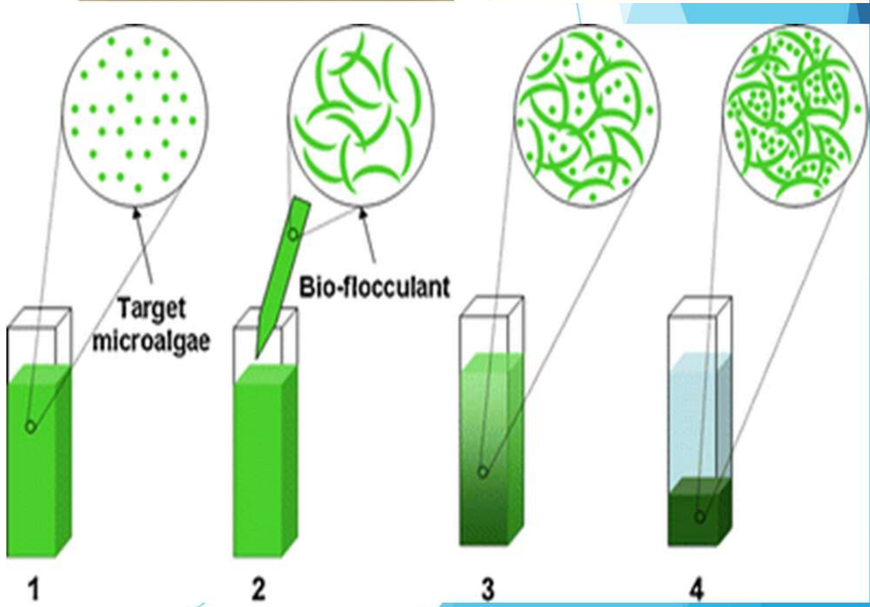
Microalgae Downstream processes

Harvesting methods for microalgal cultures:

- Bio-flocculation methods:
  - **Auto-Flocculation:** Auto-flocculation (AF) leads to co-precipitation with inorganic salt ions at high pH levels
  - **Bio-Flocculation:** bio-flocculation (BF) depends on the production of extracellular polymeric substances during algal growth phases





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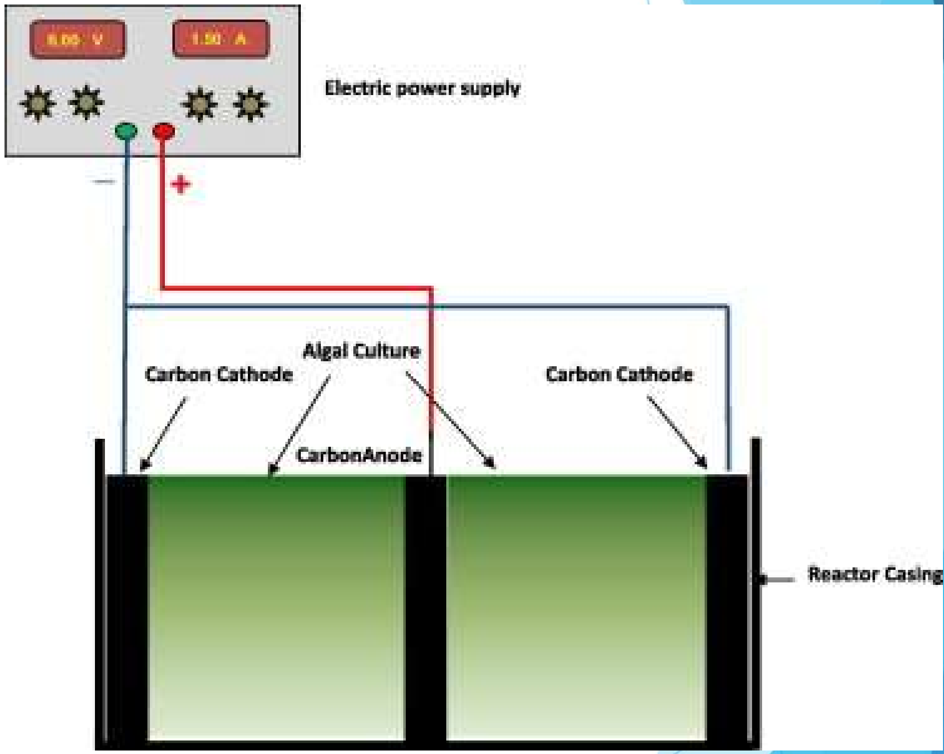
Microalgae Downstream processes

Harvesting methods for microalgal cultures:

- Electrophoresis methods:
  - **Electrolitic coagulation**
  - **Electrolitic flocculation**
  - **Electrolitic flotation**

- https://youtu.be/1I85J9dU5fY



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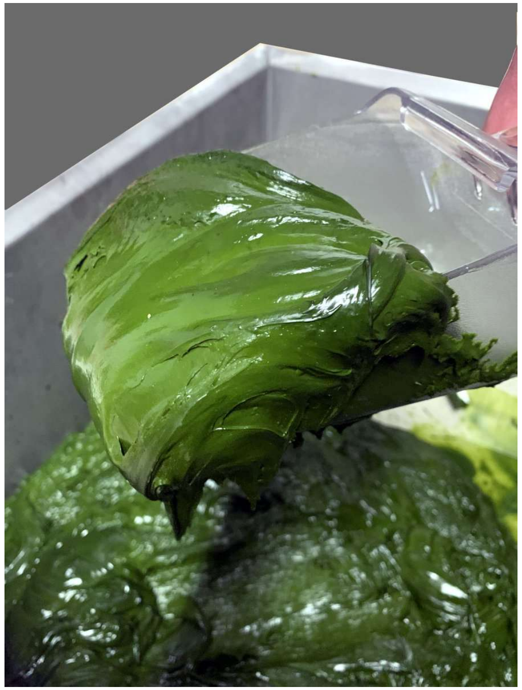
Microalgae Downstream process ➔ whole crude concentrates

Live paste ➔ aquaculture larvae feed (75% moisture content)



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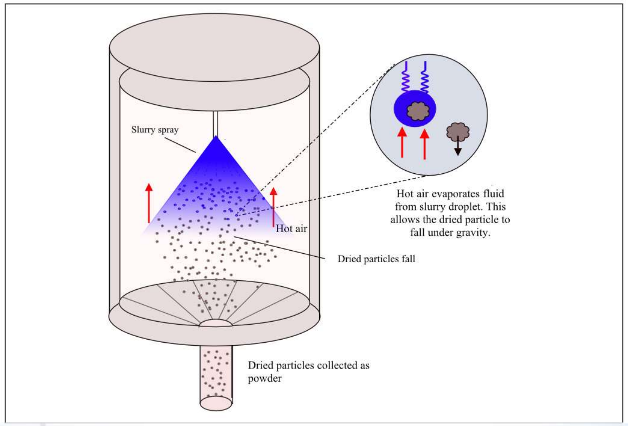
Microalgae Downstream process → dehydrated crude concentrates

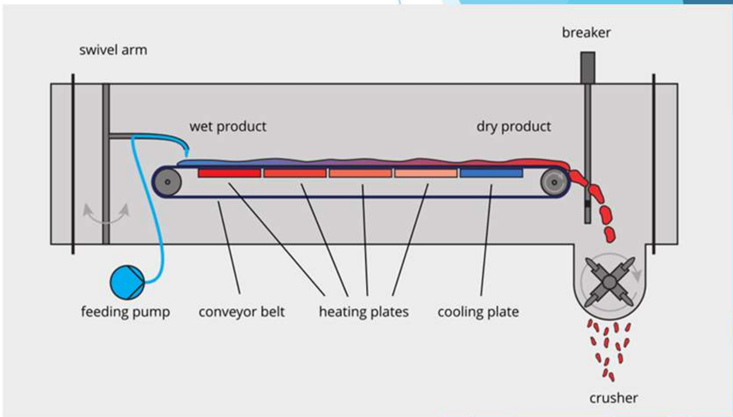
→

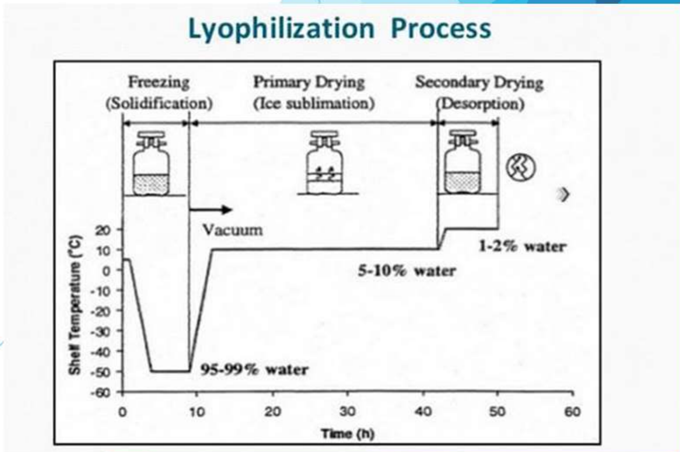
Microalgae Downstream processes

Dehydrating methods for microalgal cultures: (Goal: <10% moisture content)

- Spray drying.
- Solar drying
- Convective hot air drying
- Belt vacuum drying
- Lyophilization







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Microalgae Downstream process ➔ dehydrated crude concentrates

Powder ➔ food, feed, cosmetic applications

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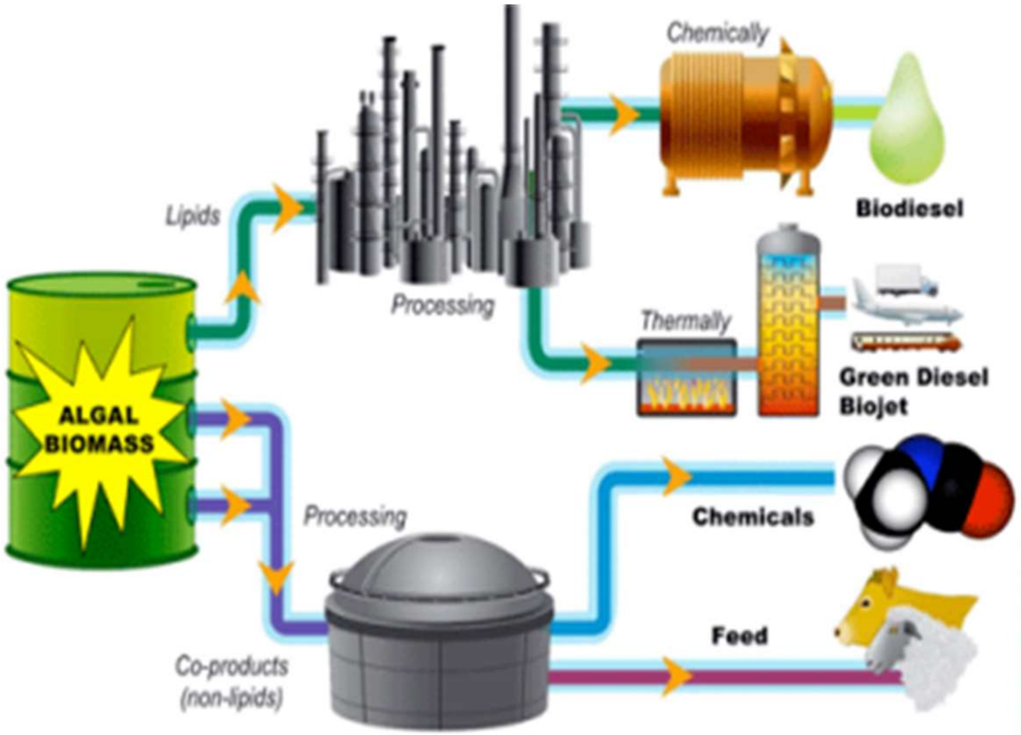
Microalgae Downstream process ➔ Biorefinery concept



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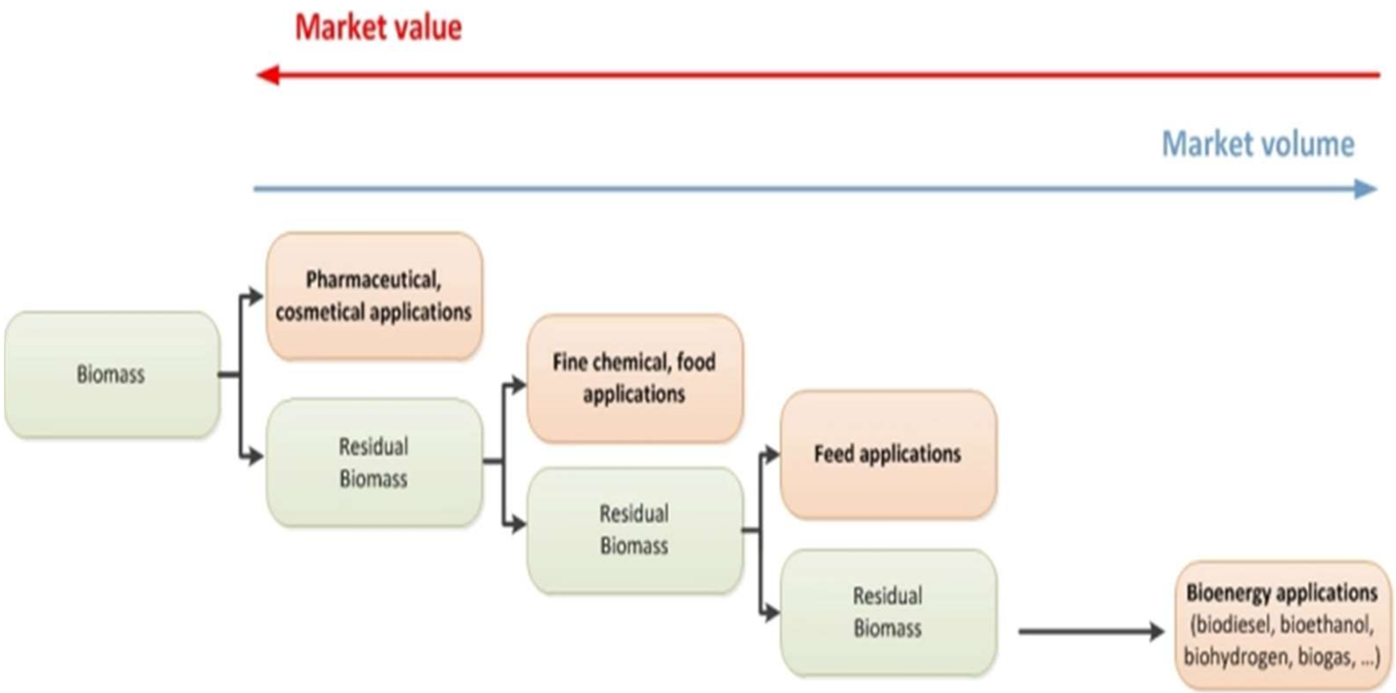
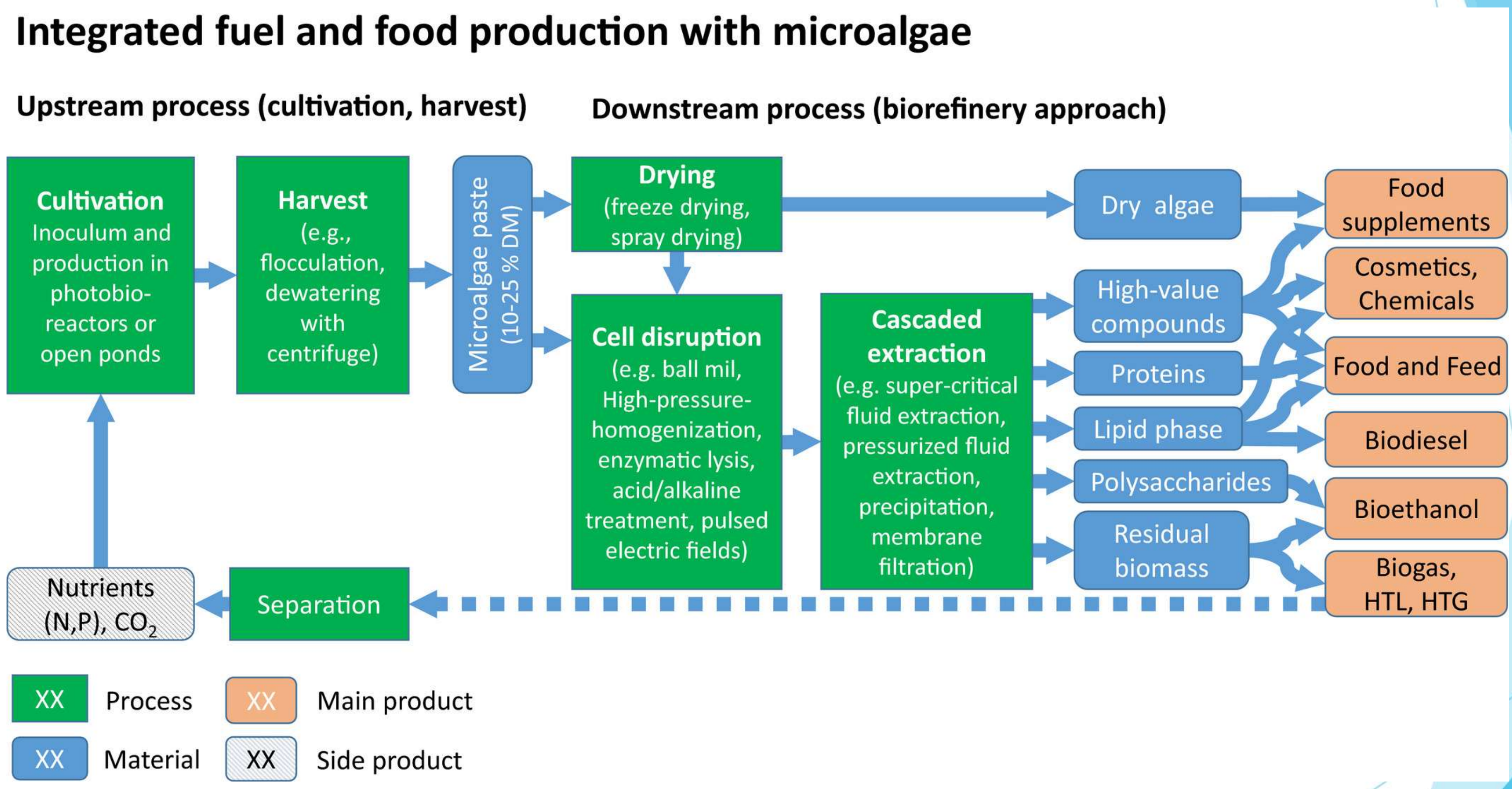
Microalgae Downstream process ➔ Biorefinery concept

Biorefinery refers to a chemical facility that carries out a series of integrated processes with the purpose of profitably and sustainably fractionating renewable algal or terrestrial biomass into a plethora of intermediate and final products, primarily biofuels and bioproducts, for use in the economy.



A microalgae biorefinery concept based on the cascading principle

Microalgae Downstream process ➔ Biorefinery concept



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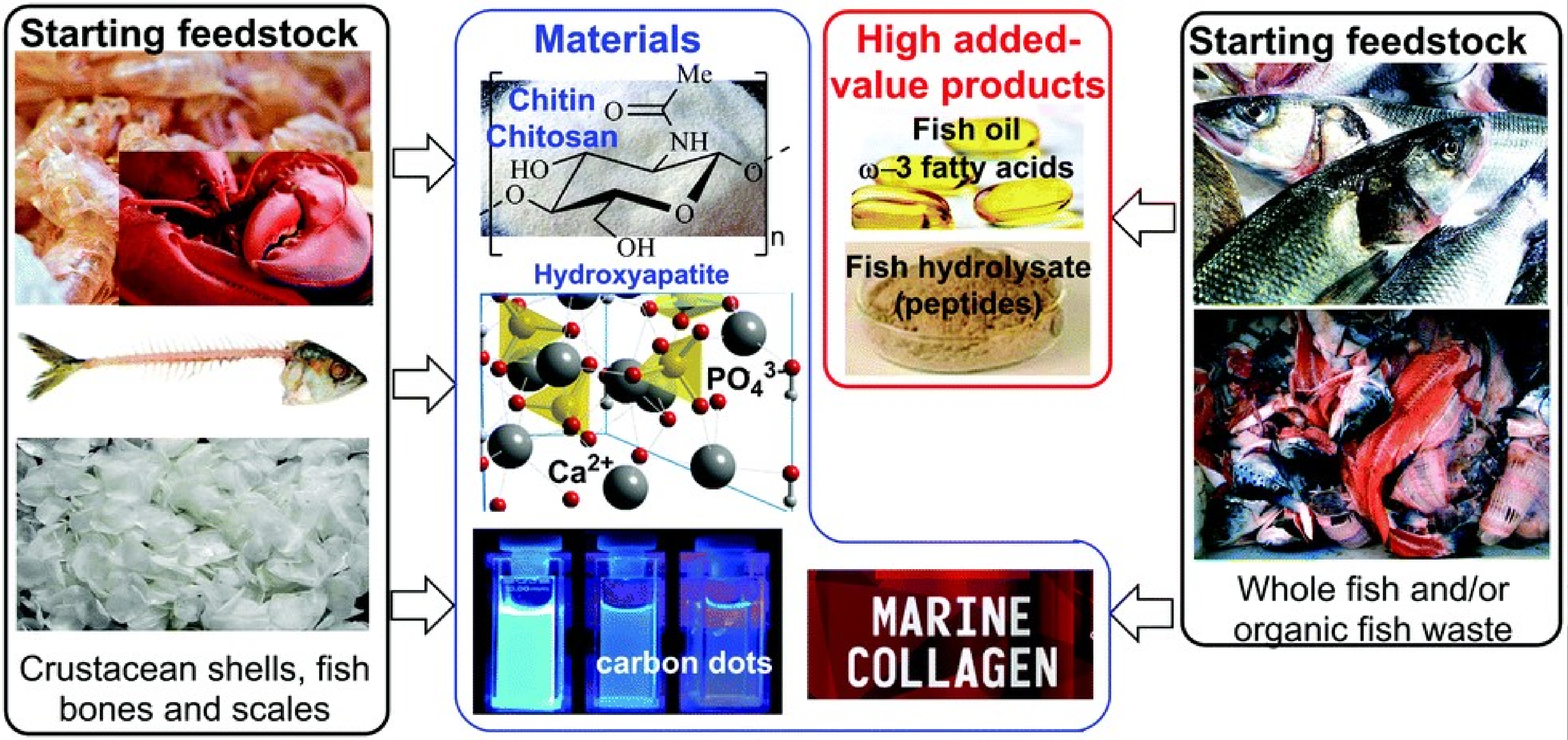
Seafood waste conversion

Seafood waste conversion ➔ Biorefinery concept



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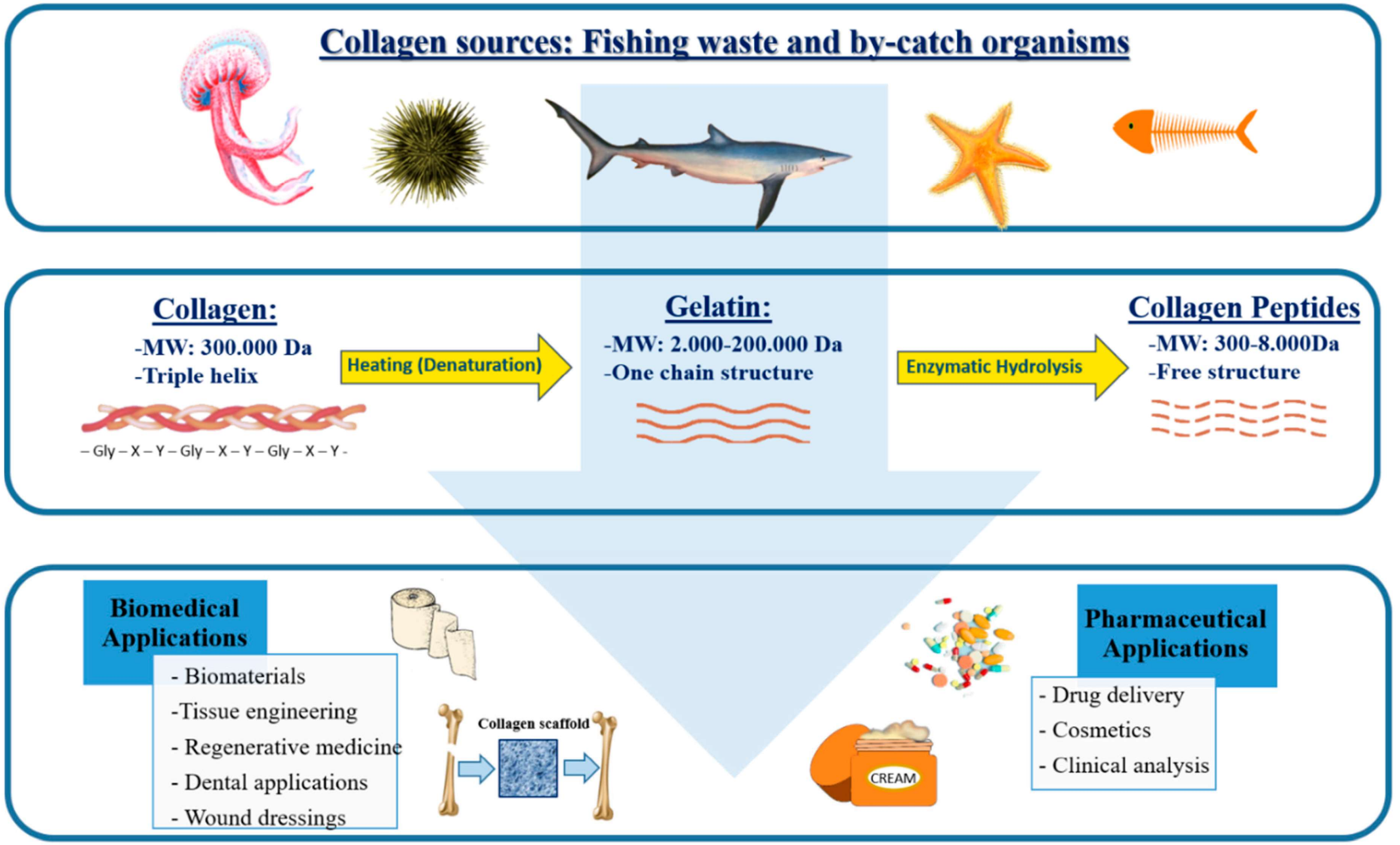
Seafood waste conversion ➔ Biorefinery concept



Seafood waste conversion → Biorefinery concept

SHELL BIOREFINERY

Crustacean shells contain three primary chemicals that have many industrial uses. Developing a sustainable way to refine them could add billions of dollars to the bioeconomy.

Shell waste

Fractionation

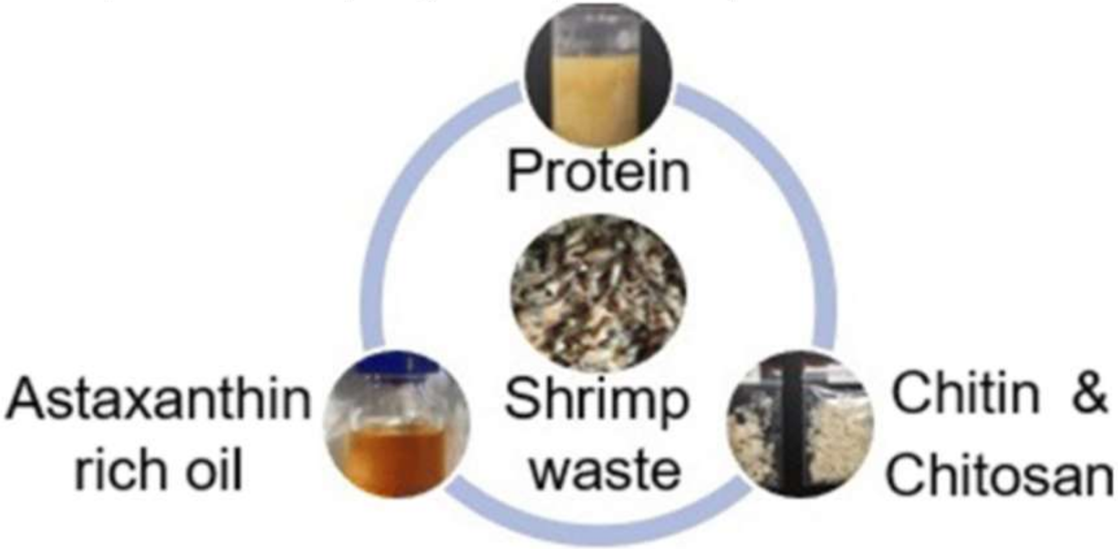
Product	Use
Calcium carbonate (20–50%)	Pharmaceutical, agricultural, construction and paper industries — including pigments, fillers, soil treatments, rubber and plastics.
Chitin (15–40%)	Nitrogen-rich chemicals for pharmaceuticals, cosmetics, textiles, water treatment, household cleansers, soaps, carbon dioxide sequestration.
Protein (20–40%)	Fertilizers and animal feeds.

Seafood waste conversion ➔ Biorefinery concept

A step to shell biorefinery—Extraction of astaxanthin-rich oil, protein, chitin, and chitosan from shrimp processing waste

Biomass Conversion and Biorefinery (IF 2.602) **Pub Date : 2020-11-02** , *DOI: 10.1007/s13399-020-01074-5*

P. A. Aneesh, R. Anandan, Lekshmi R. G. Kumar, K. K. Ajeeshkumar, K. Ashok Kumar, Suseela Mathew



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Seafood waste conversion ➔ Biorefinery concept

Crabs

Shrimps

Chemical extraction

Griding

Biological extraction

Dilute aqueous HCL solution

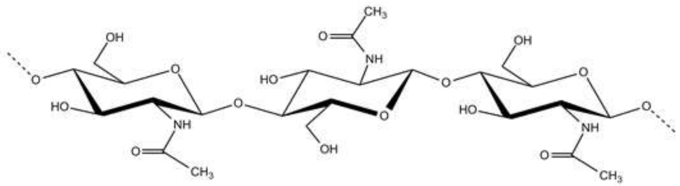
Decalcification

Deproteination

Proteases producing bacteria

Decolorization

Chitin

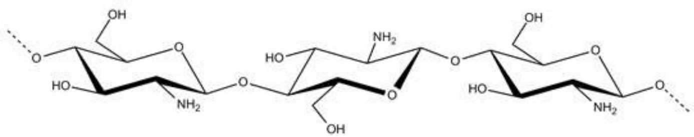


Hot concentrated NaOH solution (40-50%)

Deacetylation

Chitin deacetylase

Chitosan



AquaVIP Klaipeda - Innovative Aquaculture Summer School
Klaipeda University – Klaipeda Science & Technology Park

Blue Biotechnology Pipeline: From Discovery to Application

Tuesday, June 29th 2021

Microalgae & Fish Waste

Thank you!!!!!

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Jerónimo Chirivella Martorell PhD.