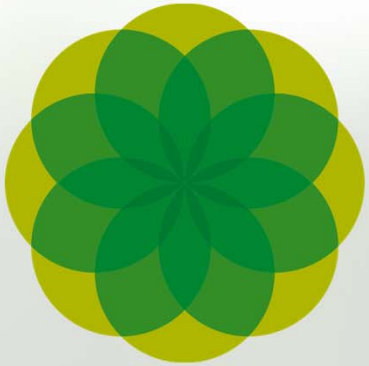


ENHANCE

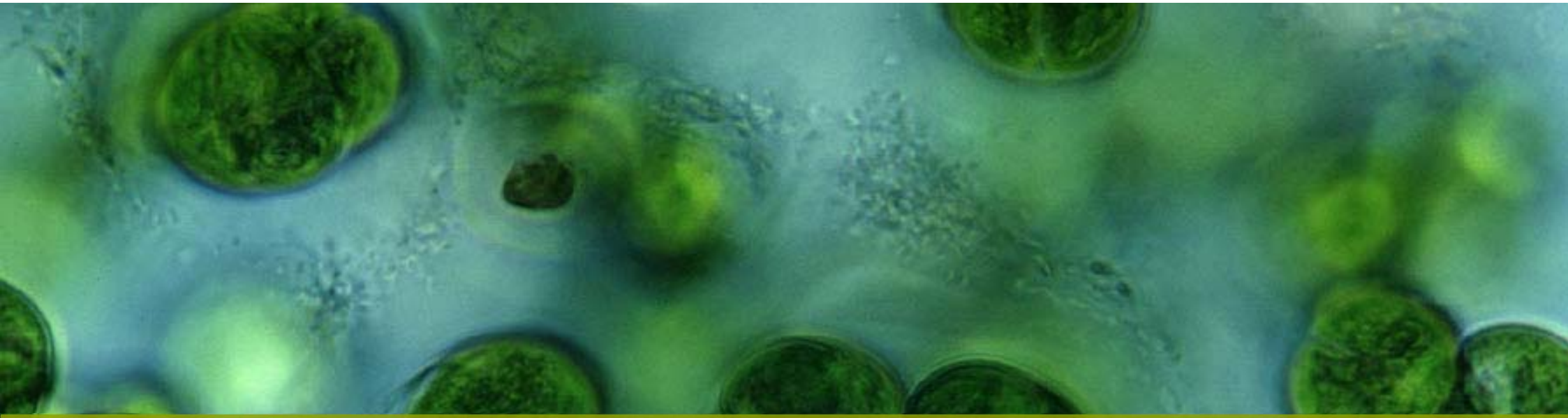
MICROALGAE

EnhanceMicroAlgae Project

High added-value industrial opportunities for microalgae in the Atlantic Area

Asterio Sánchez Mirón. University of Almería

La Rochelle, 10/20/2022



Microalgae-based bioprocesses and bioproducts

Marine microalgae Biotechnology Research Group

- Chemical Engineering Department
- Engineering treatment of bioprocesses

Members: **21**

Ph.D.: 16

Master: 5

Since late 1980s

>410 Papers in indexed Journals

44 Books/book chapters

> 440 Congress communications

> 180 Thesis/Master Thesis

58 Contracts (private and public organizations)

100 Research Projects

16 Patents

- Laboratory and pilot scale facilities to produce microalgal biomass indoor or outdoor using open and closed photobioreactors
- Technologies for biomass harvesting, dewatering and downstream processing
- Equipment for extraction, fractionation and quantification of biomolecules from microalgae

i. Industrial exploitation of microalgae for fine chemicals and food/feed ingredients (PUFAs, antioxidants, and other biomass fractions) (FI).

ii. Use of different effluents for growing microalgae.

iii. Photobioreactor (PBR) engineering and scale up (including anti-biofouling materials).

iv. Computational Fluid Dynamics (CFD) for PBR improvement.

v. Downstream and biorefinery-based production strategy.

vi. Life-Cycle Assessment (LCA) and techno-economic analysis (TCA) of microalgal processes.

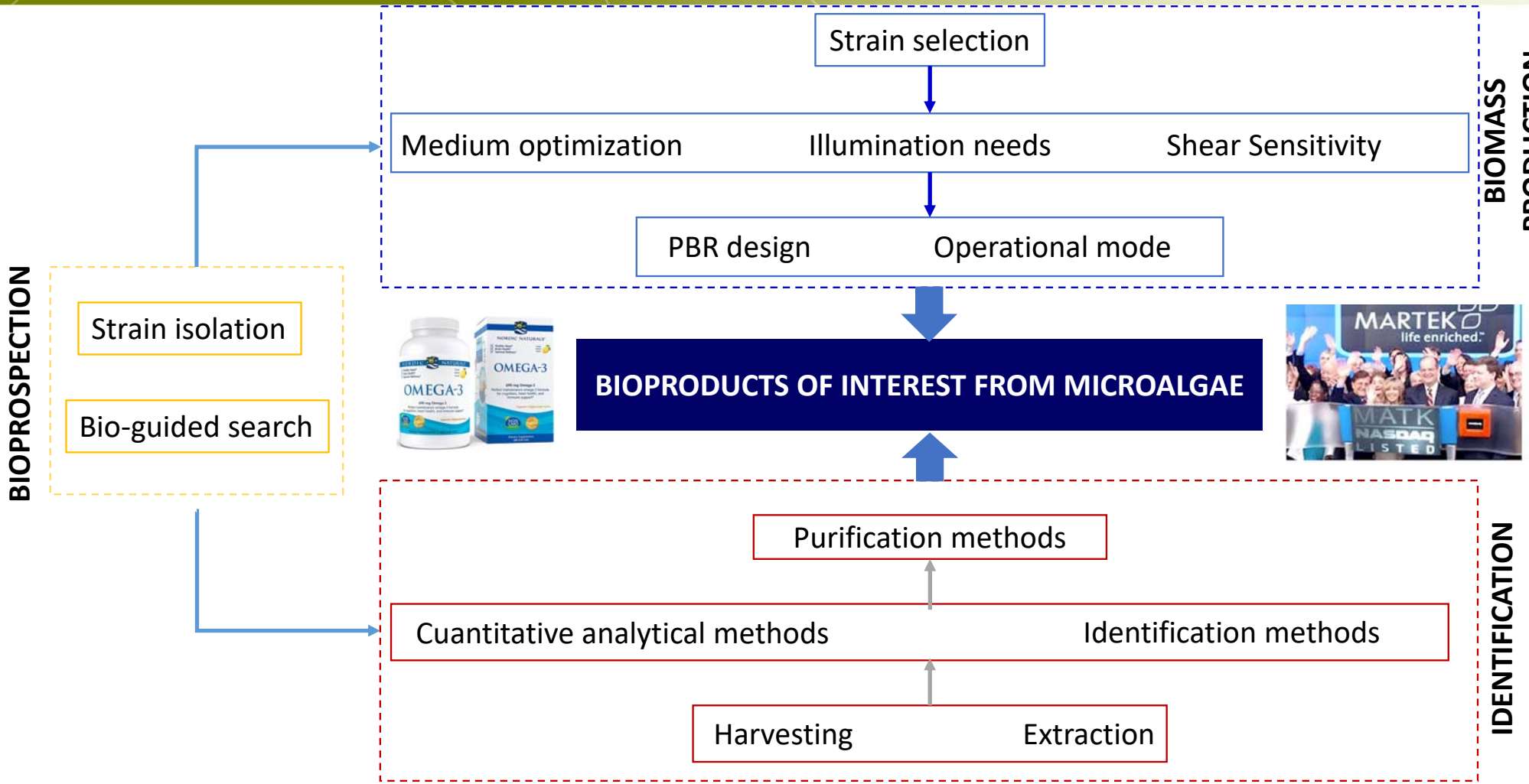
vii. Production of high value lipids for food ingredients / bioactives (Long chain PUFA concentrates from marine oils; Polar lipid concentrates from microalgae oils, Tailored lipids by enzyme technology from vegetable / marine oils)

viii. Bio-based screening programs for biodiscovery.

ix. Bioprocess development for marine dinoflagellates and non-conventional microalgae for reference standards and medical research.

Microalgae-based bioprocesses and bioproducts

Bioprocess concept

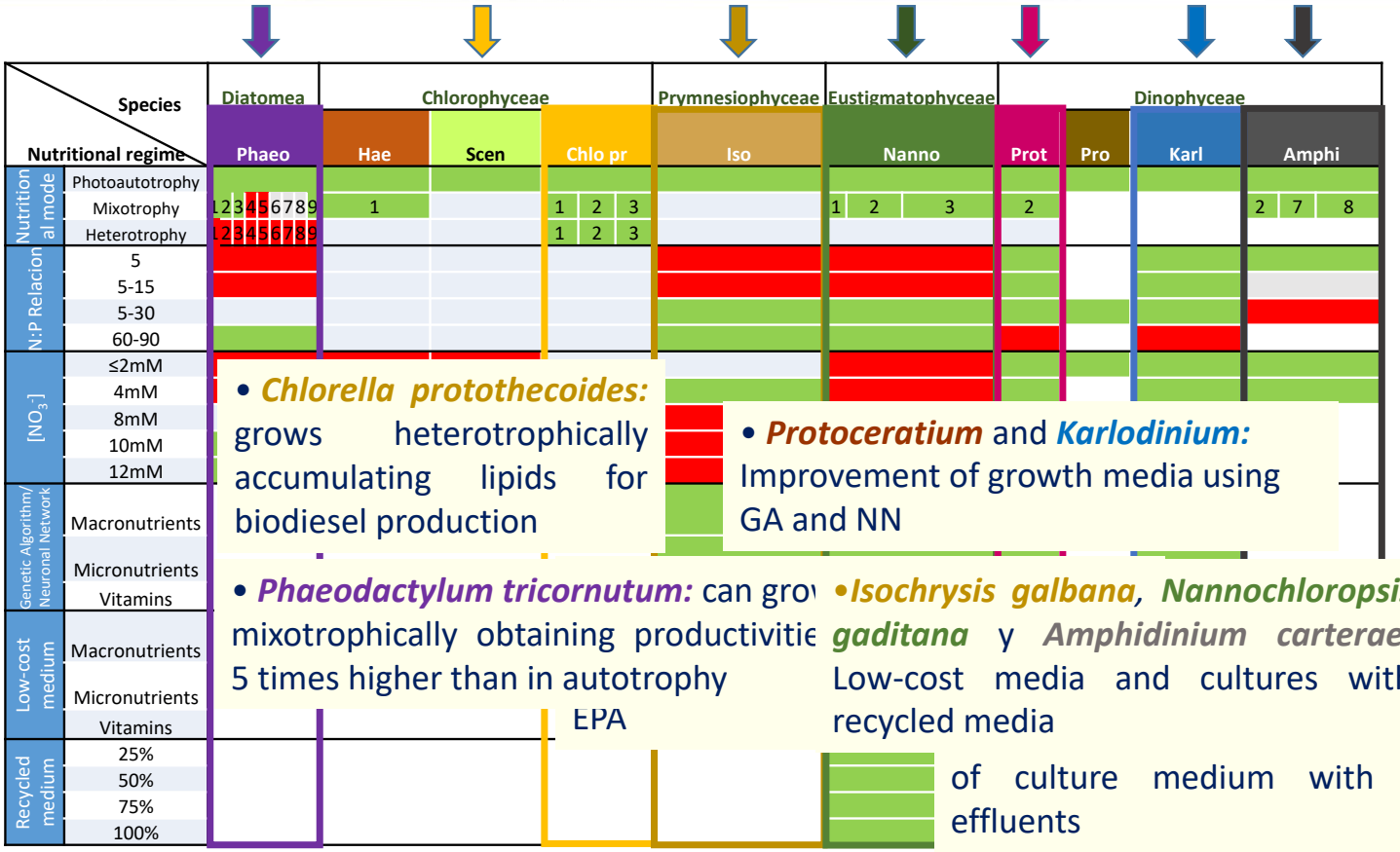


Microalgae-based bioprocesses and bioproducts

Medium optimization

Positive effect
Neutral effect
Negative effect

1.Glucose
2.Glycerol
3.Fructose
4.Lactose
5.Sacarose
6.Acetate
7.Ammonia
8.Urea
9.Ammonia+nitrate



• ***Chlorella protothecoides:*** grows heterotrophically accumulating lipids for biodiesel production

• ***Protoceratium*** and ***Karlodinium:*** Improvement of growth media using GA and NN

• ***Phaeodactylum tricornutum:*** can gro mixotrophically obtaining productivitie 5 times higher than in autotrophy

•***Isochrysis galbana***, ***Nannochloropsis gaditana*** y ***Amphidinium carterae:*** Low-cost media and cultures with recycled media

EPA

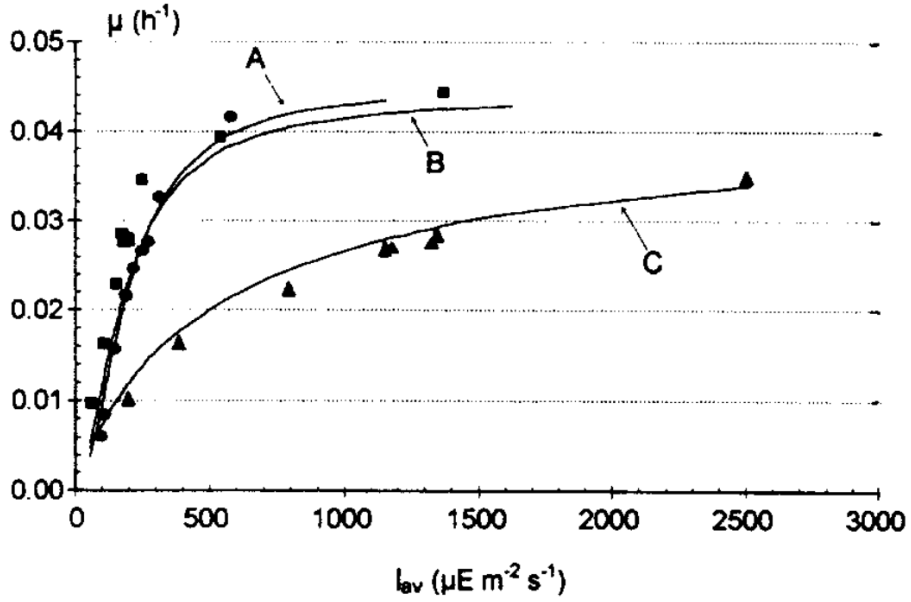
ulation of culture medium with fisheries effluents

Phaeo *Phaeodactylum tricornutum*
Hae *Haematococcus pluvialis*
Scen *Scenedesmus almeriensis*
Chlo pr *Chlorella protothecoides*
Iso *Isochrysis galbana*
Nanno *Nannochloropsis gaditana*
Prot *Protoceratium reticulatum*
Pro *Prorocentrum beliceanum*
Karl *Karlodinium veneficum*
Amphi *Amphidinium carterae*

Microalgae-based bioprocesses and bioproducts

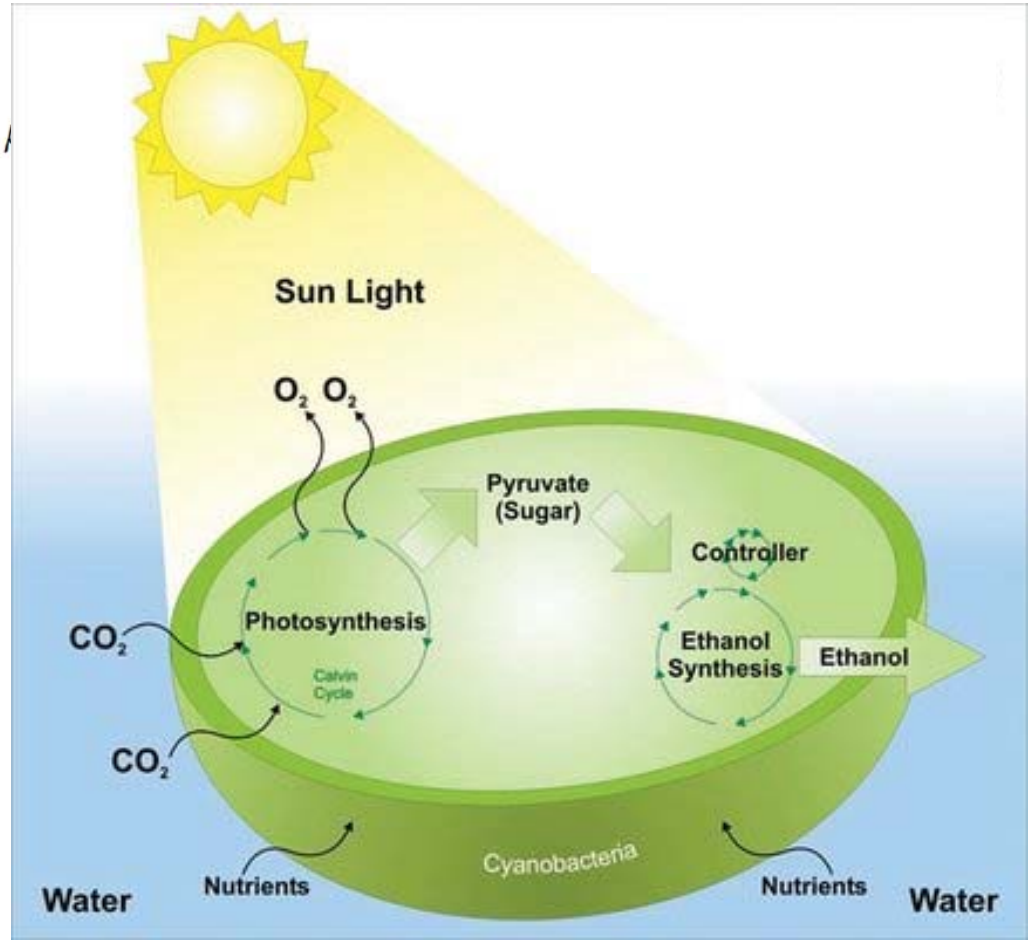
Illumination needs

Limitation and inhibition Growth Model



$$\mu = \frac{\mu_{max} I_{av}^{(n_2/I_o)}}{\left(I_k + (I_o/K_I)^{n_1}\right)^{(n_2/I_o)} + I_{av}^{(n_2/I_o)}}$$

Molina-Grima et al. (1996). J. Biotechnology 45, 59-69

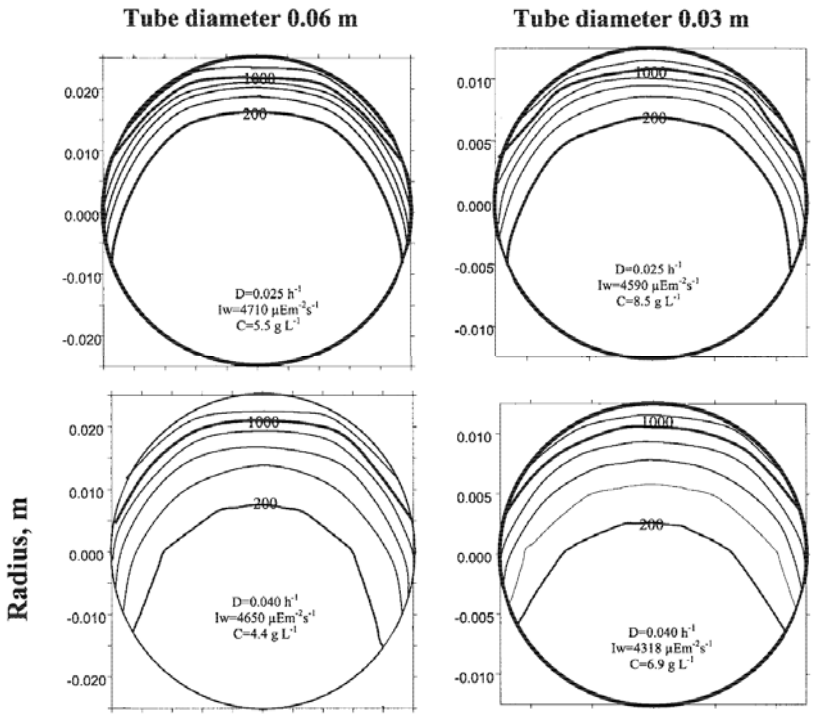


Molina Miras et al. (2018). Algal Research 31, 87-98

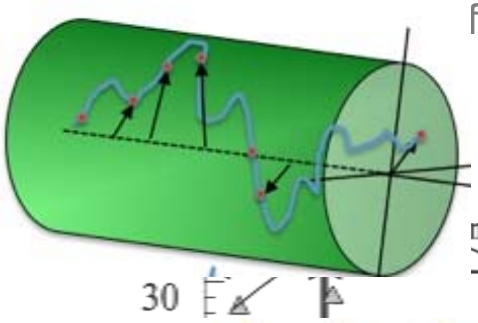
Microalgae-based bioprocesses and bioproducts

Illumination needs

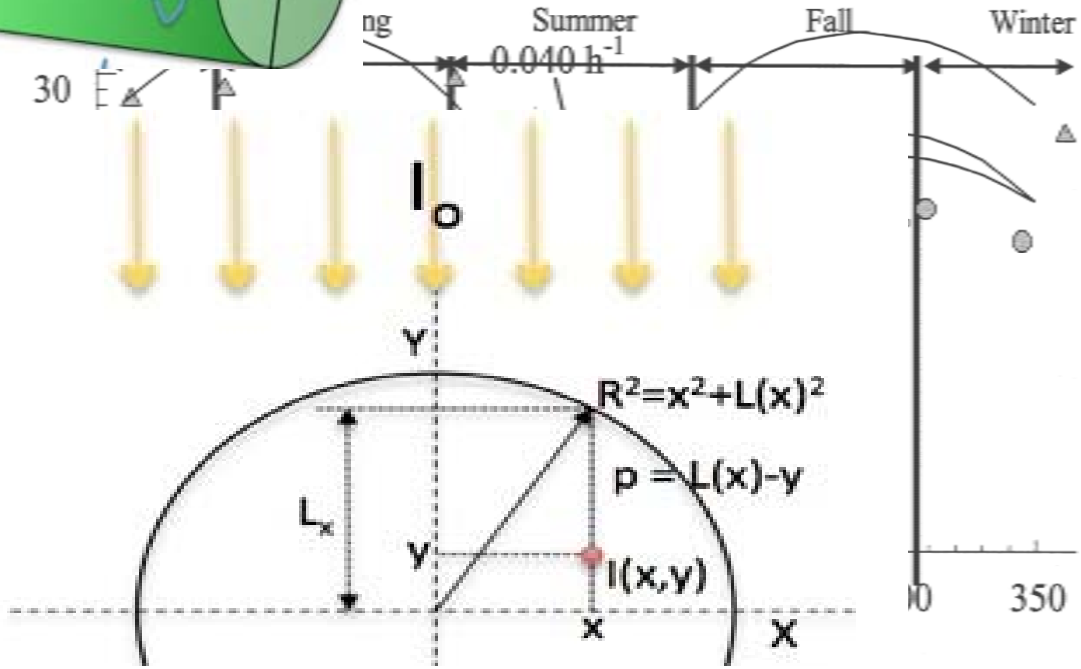
Light distribution inside PBRs



f productivities throughout the year



$$I(x,y) = I_o \cdot e^{-k_a \cdot C_b \cdot (\sqrt{R^2 - x^2} - y)}$$

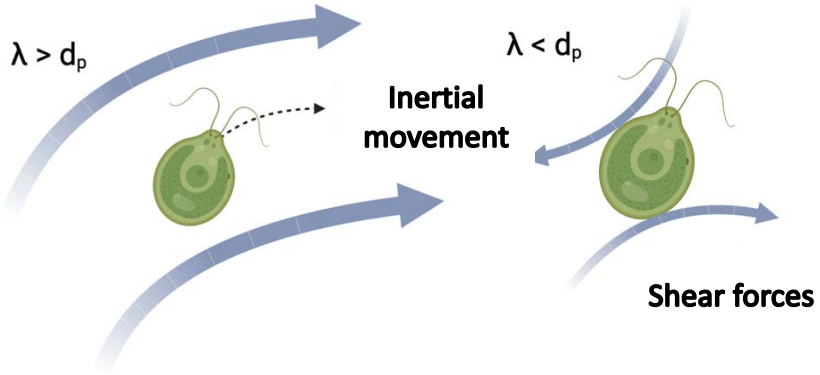


Acién-Fernández et al. (1999). Bio&Bio 68, 173-183

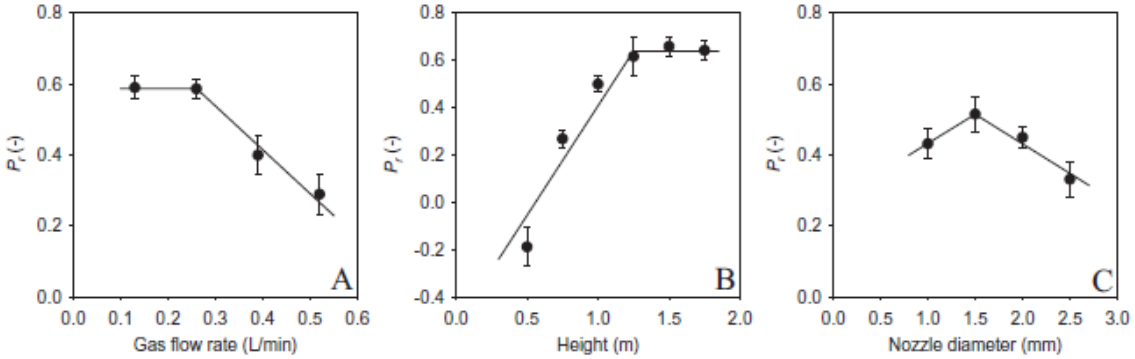
Microalgae-based bioprocesses and bioproducts

Shear Sensitivity

Influence of PBR design and operational conditions

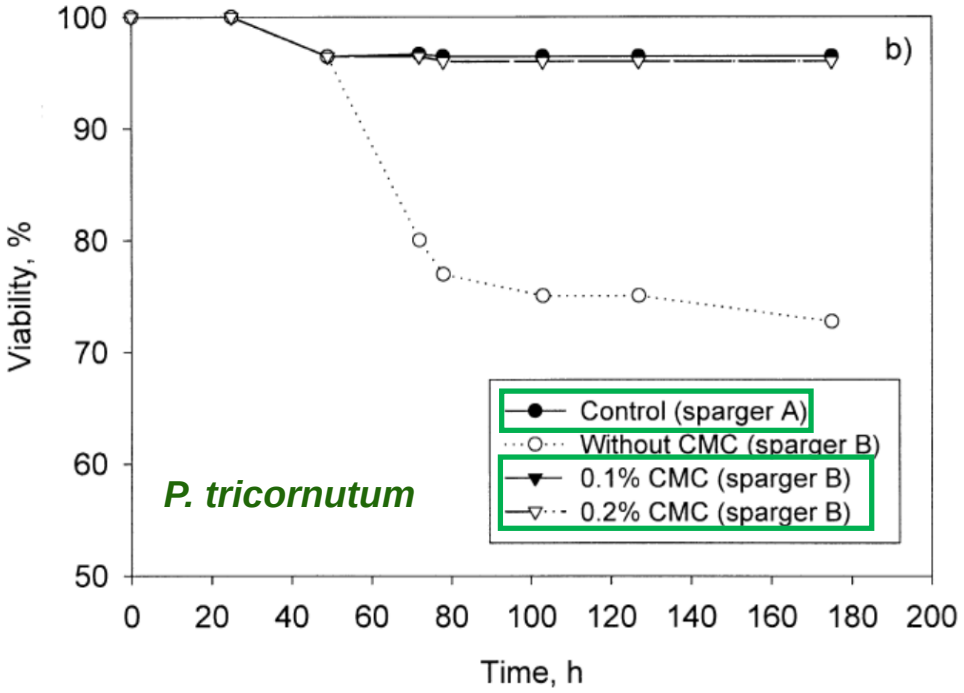


K. veneficum



López-Rosales et al. (2015). Biores. Technol. 197, 375-382

Alleviation by the use of protectants

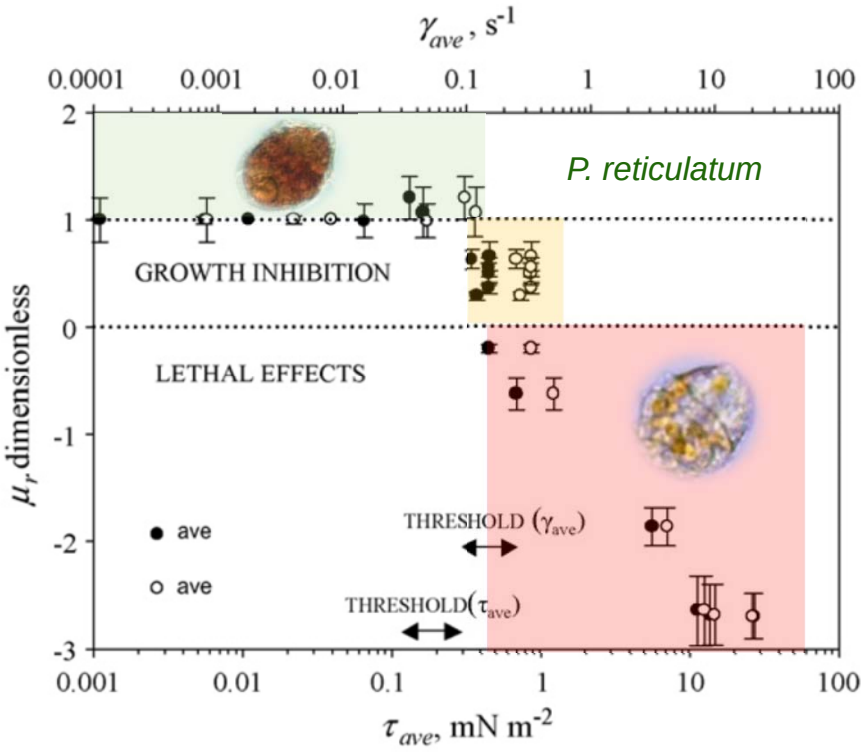


García-Camacho et al. (2001). Enz. Microb. Technol. 29, 602-610

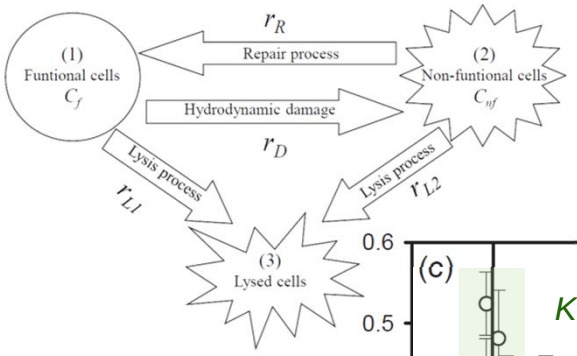
Microalgae-based bioprocesses and bioproducts

Shear Sensitivity

Protocols to characterize shear sensitivity

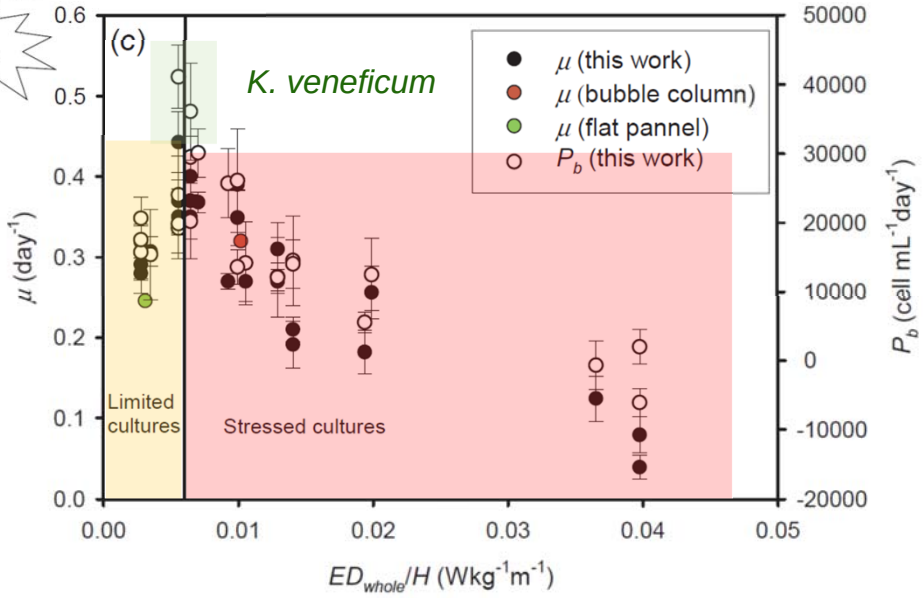


García-Camacho et al. (2007). Proc. Biochem 29, 602-610



Model for cell damage

Evaluation in different PBRs

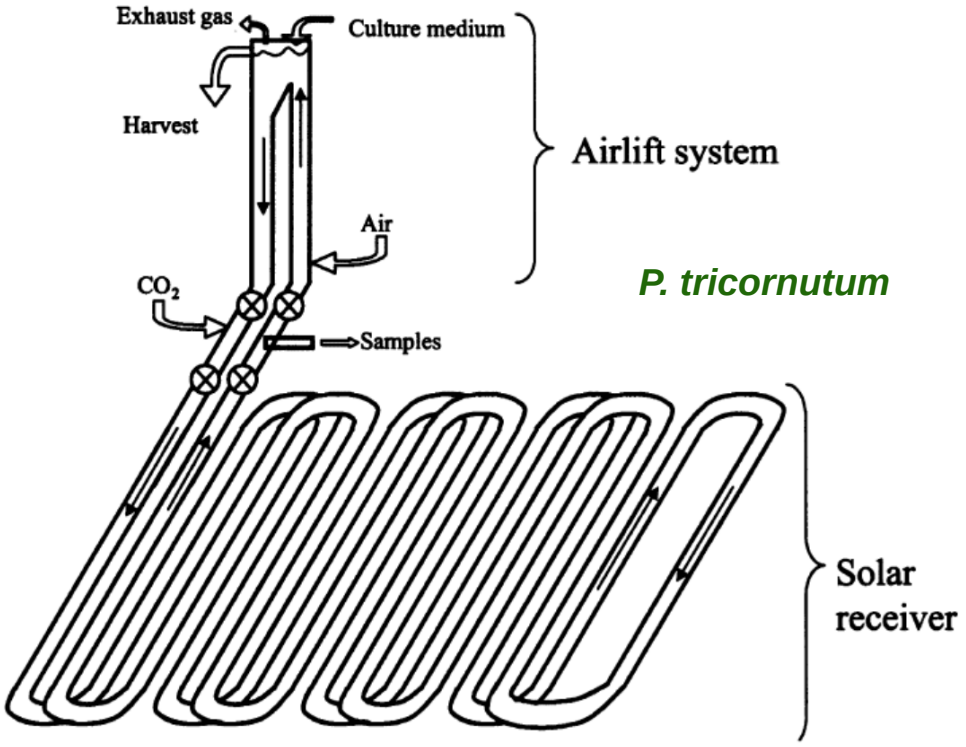


López-Rosales et al. (2019). Biores. Tech. 275, 1-9

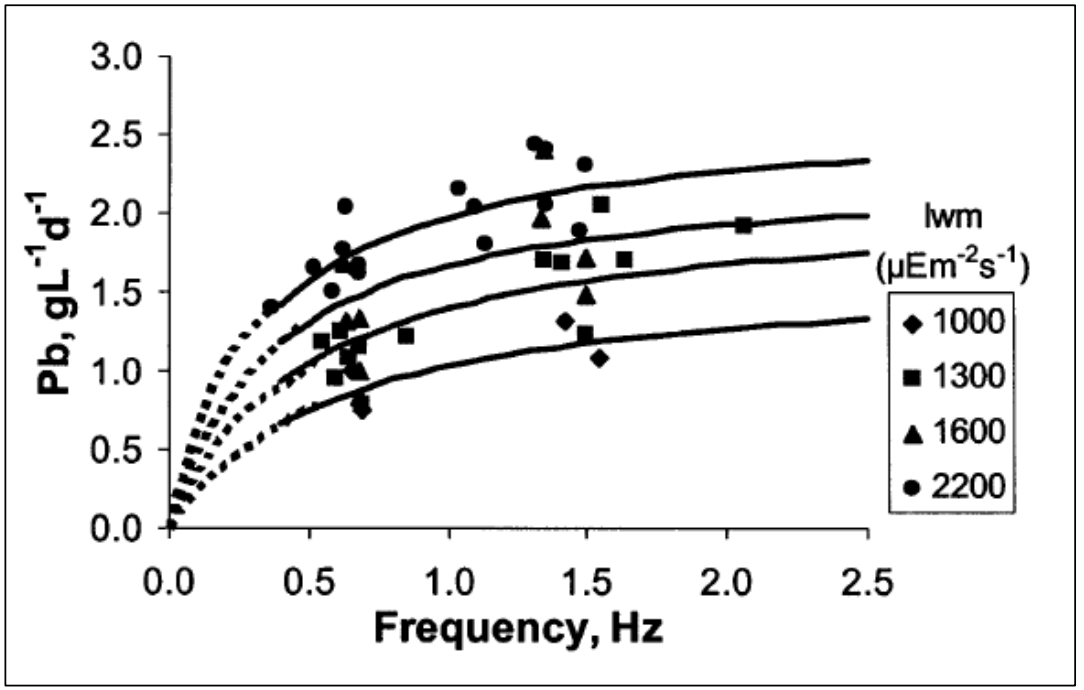
Microalgae-based bioprocesses and bioproducts

Photobioreactor design

Tubular PBRs



P. tricornutum

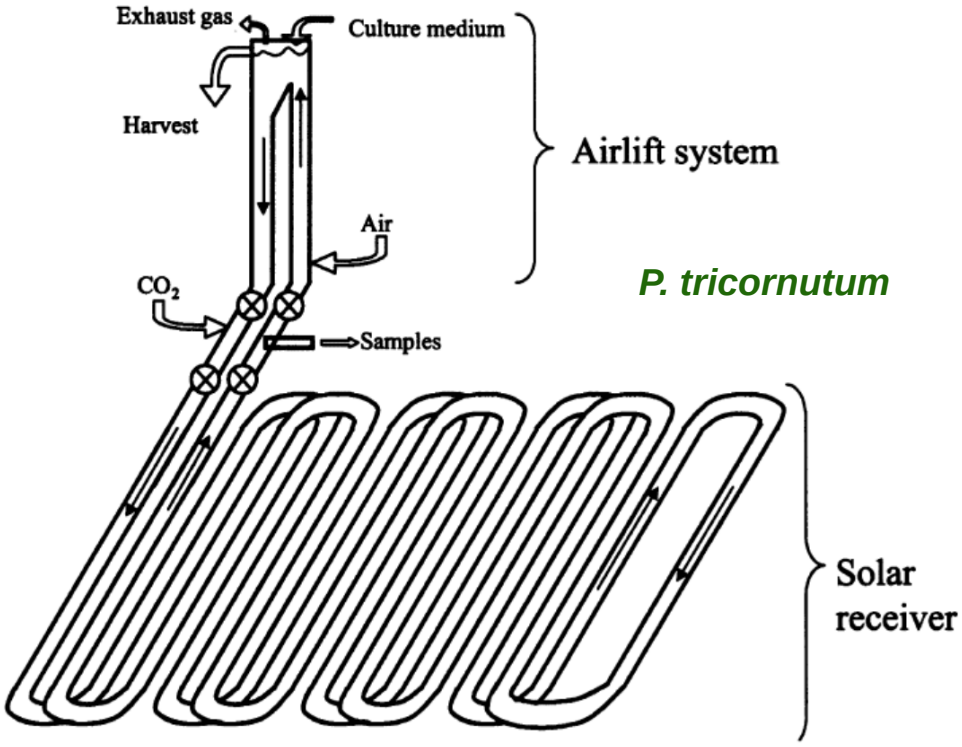


Molina-Grima et al. (2001). J. Biotechn. 92, 113-131

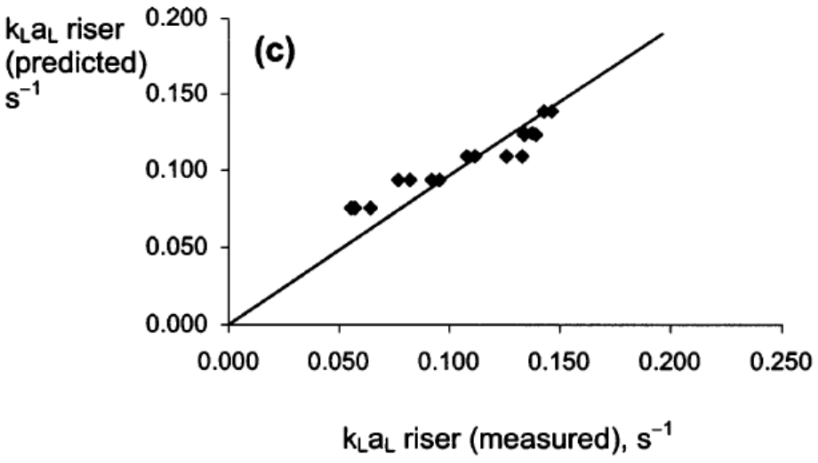
Microalgae-based bioprocesses and bioproducts

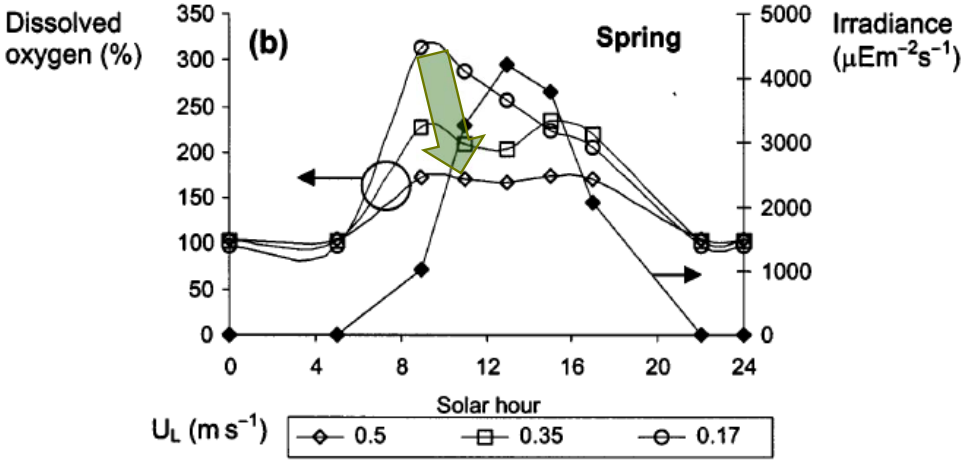
Photobioreactor design

Tubular PBRs



P. tricornutum



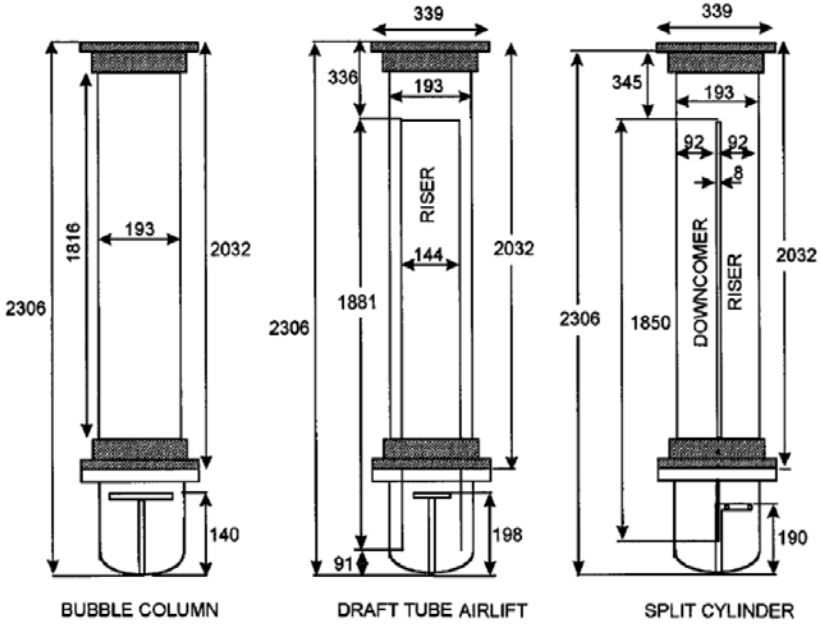


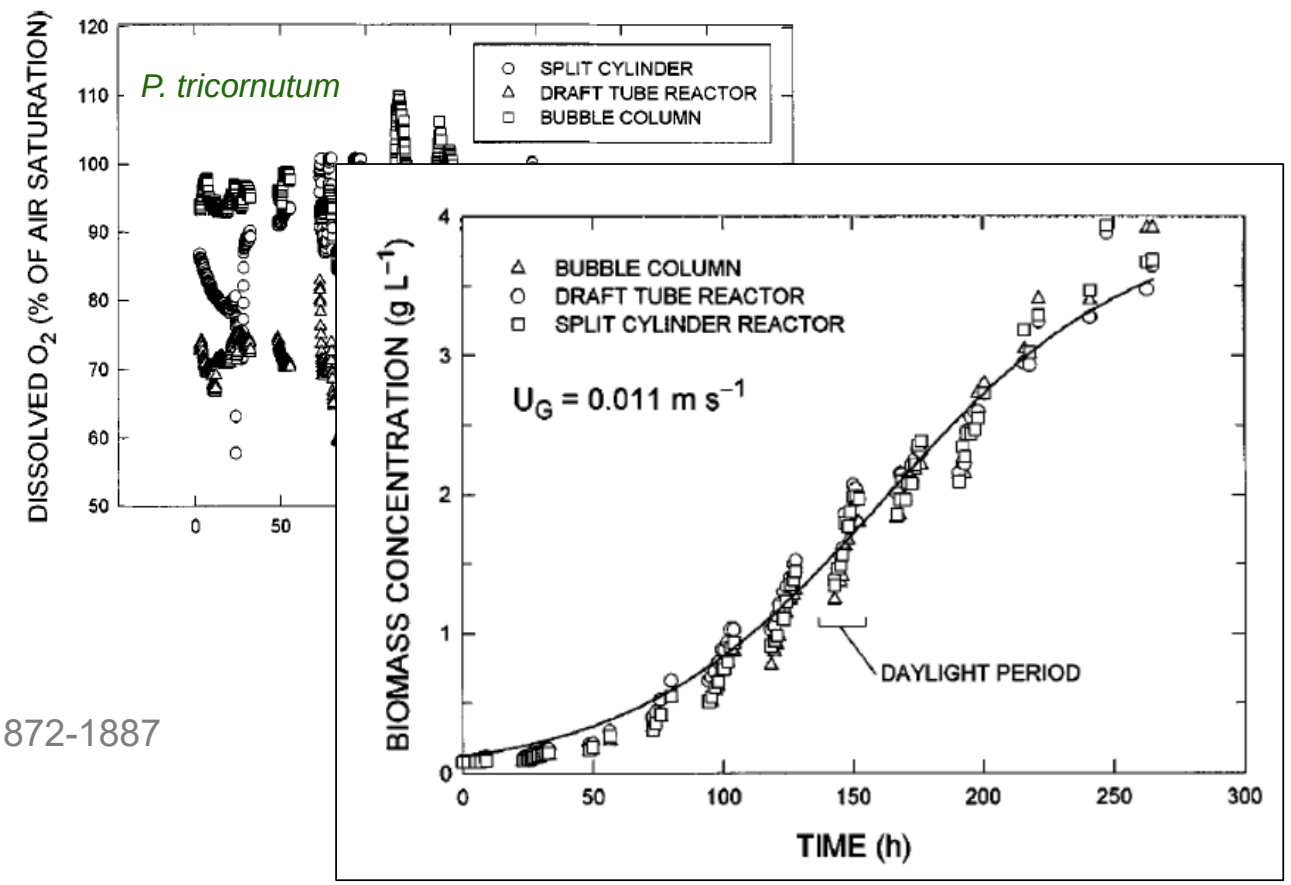
Molina-Grima et al. (2001). J. Biotechn. 92, 113-131

Microalgae-based bioprocesses and bioproducts

Photobioreactor design

Airlift vertical PBRs

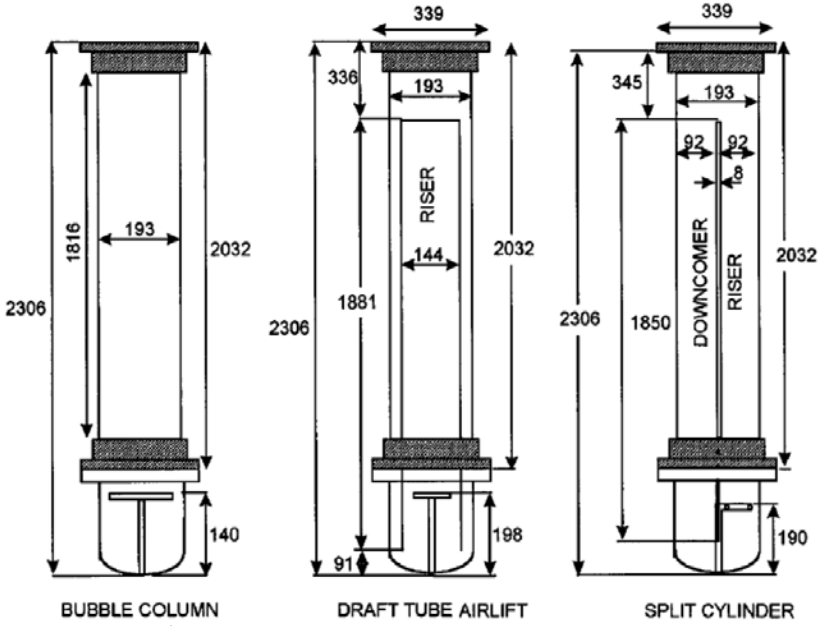




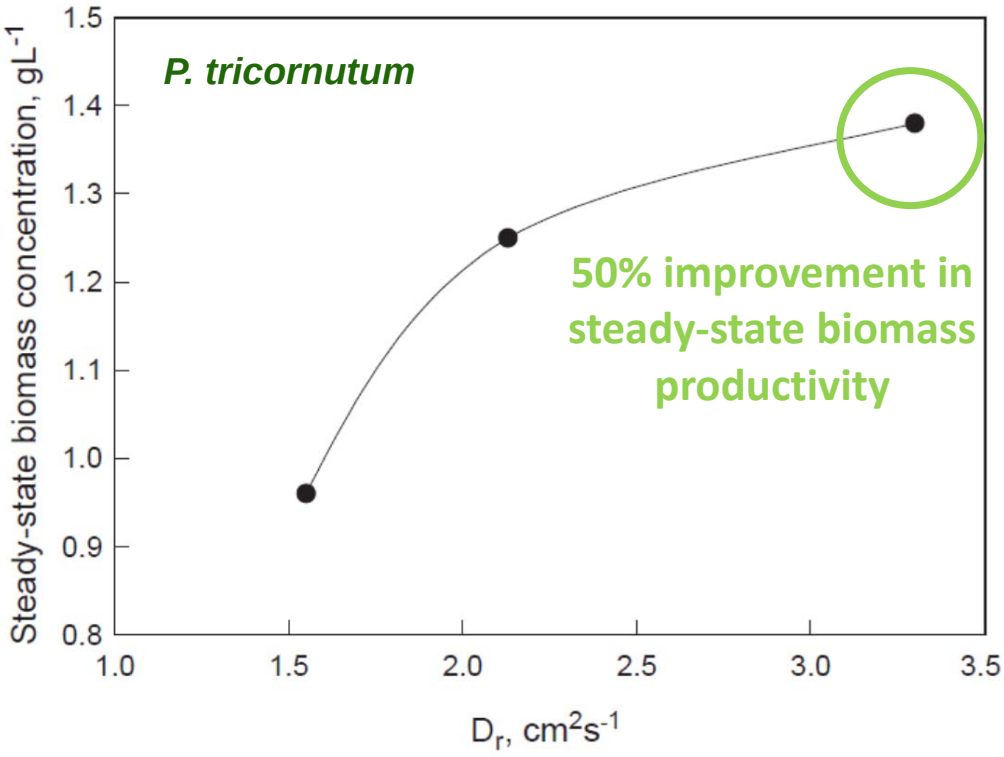
Sánchez-Mirón et al. (2000). Aiche J. 46(9), 1872-1887

Microalgae-based bioprocesses and bioproducts

Photobioreactor design



Airlift vertical PBRs



BUBBLE COLUMN RADIAL DISPERSION

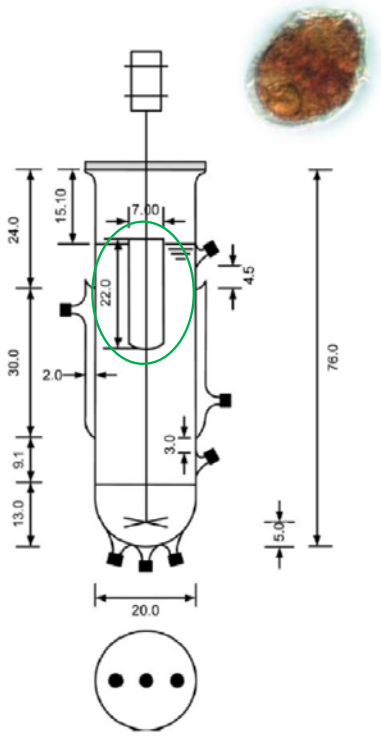
$$\frac{\partial C}{\partial t} = D_z \frac{\partial^2 C}{\partial z^2} - \frac{U_L}{\varepsilon_L}\frac{\partial C}{\partial z} + \frac{D_r}{r}\frac{\partial C}{\partial r} + D_r \frac{\partial^2 C}{\partial r^2}$$

Camacho-Rubio et al. (2004). Chem. Eng. Sci. 59, 4369-4376

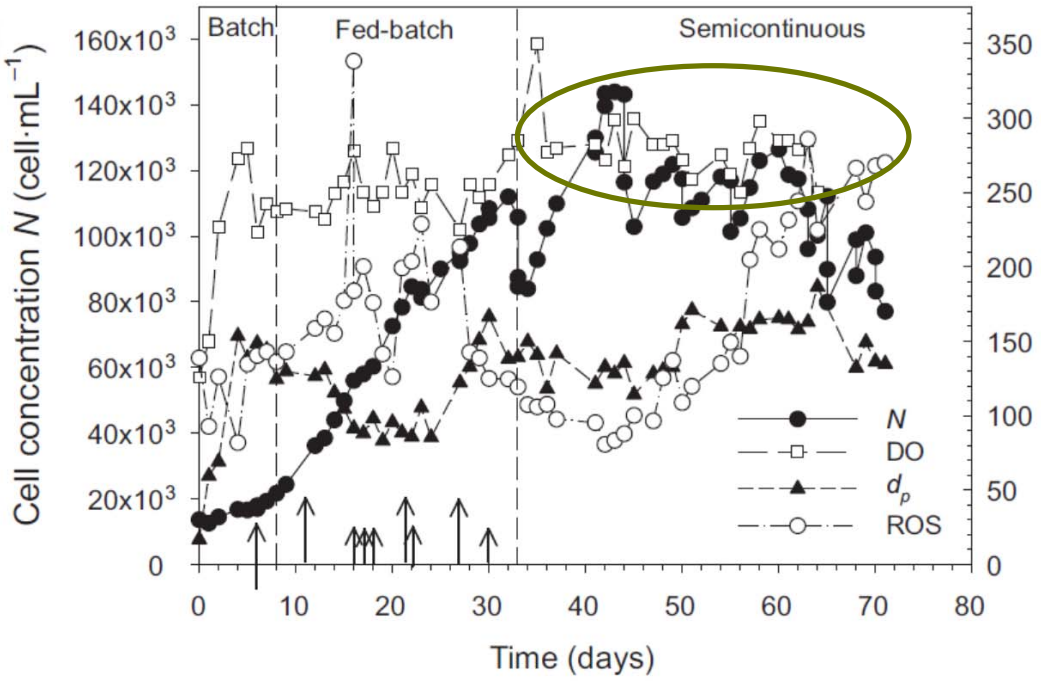
Microalgae-based bioprocesses and bioproducts

Photobioreactor design

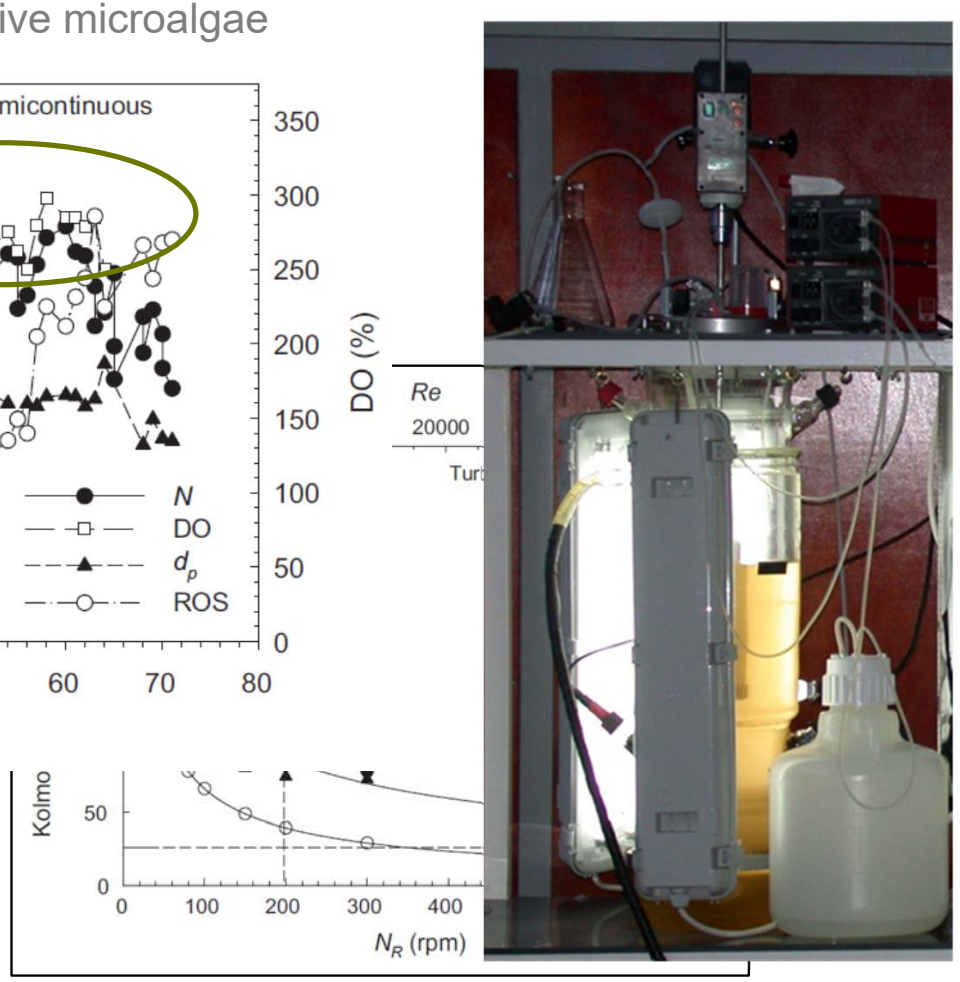
P. reticulatum



Very shear-sensitive microalgae



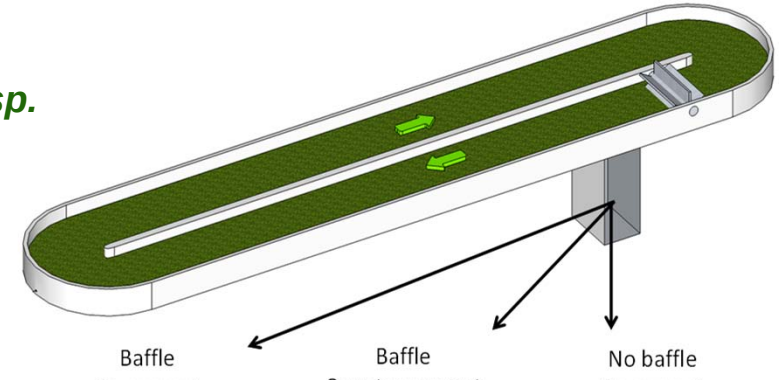
García-Camacho et al. (2011). Proc. Biochem. 46, 936-944

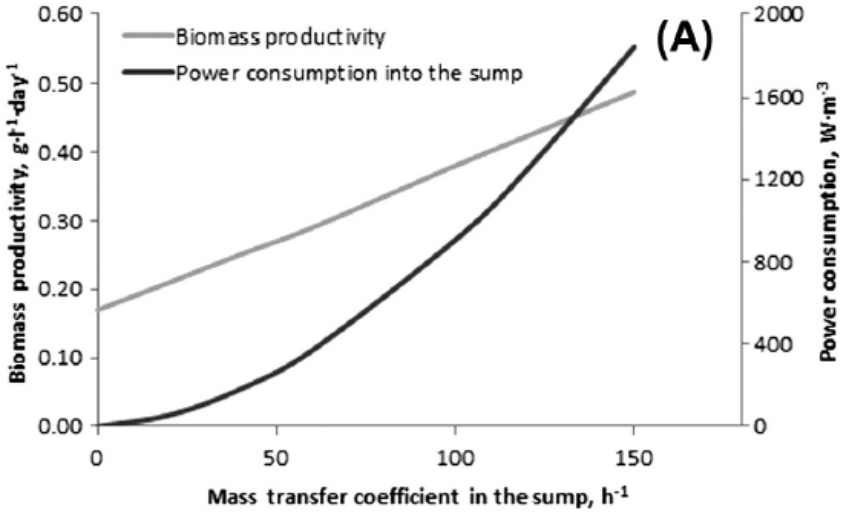


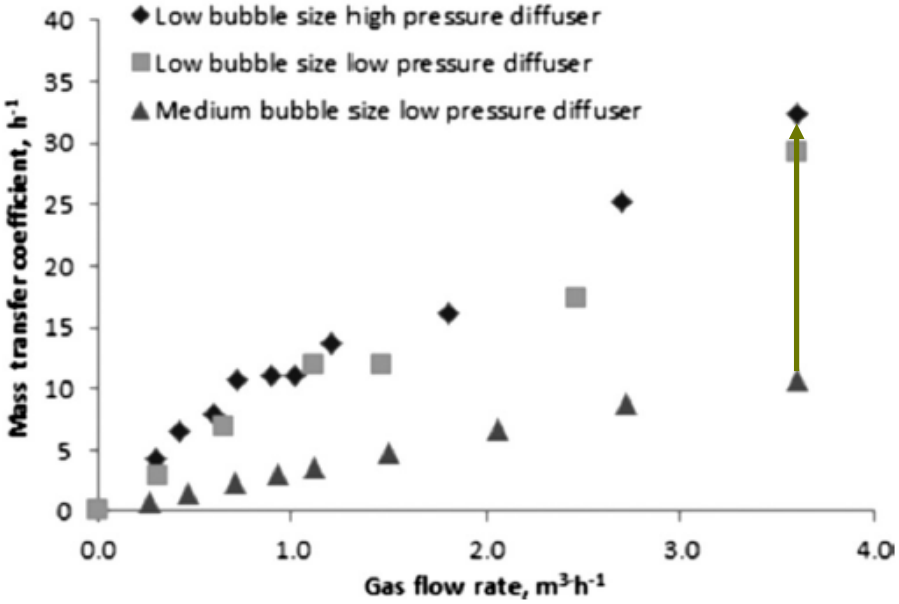
Microalgae-based bioprocesses and bioproducts

Photobioreactor design

Chlorella sp.





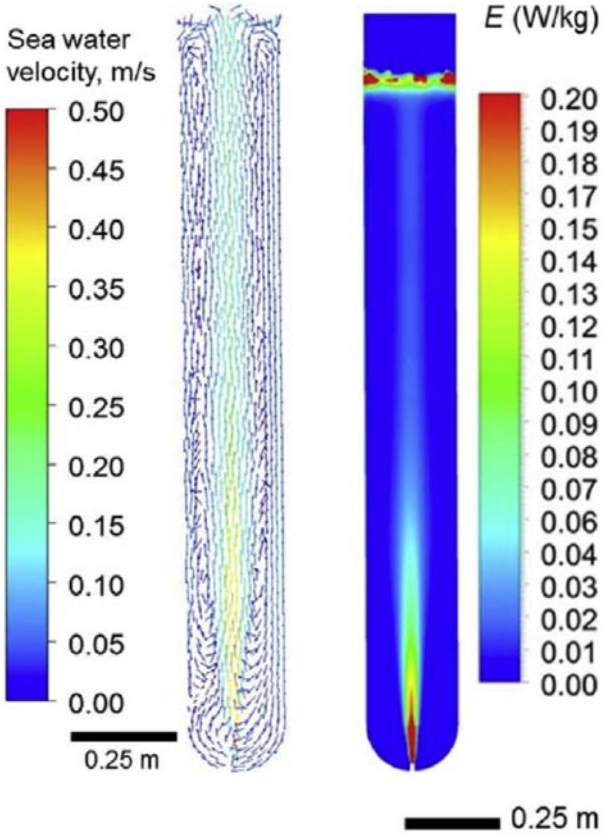


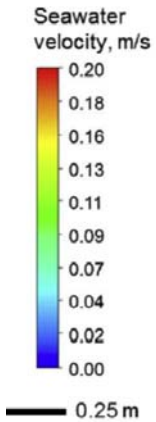
Mendoza et al. (2013). Biores. Techn. 137, 188-195

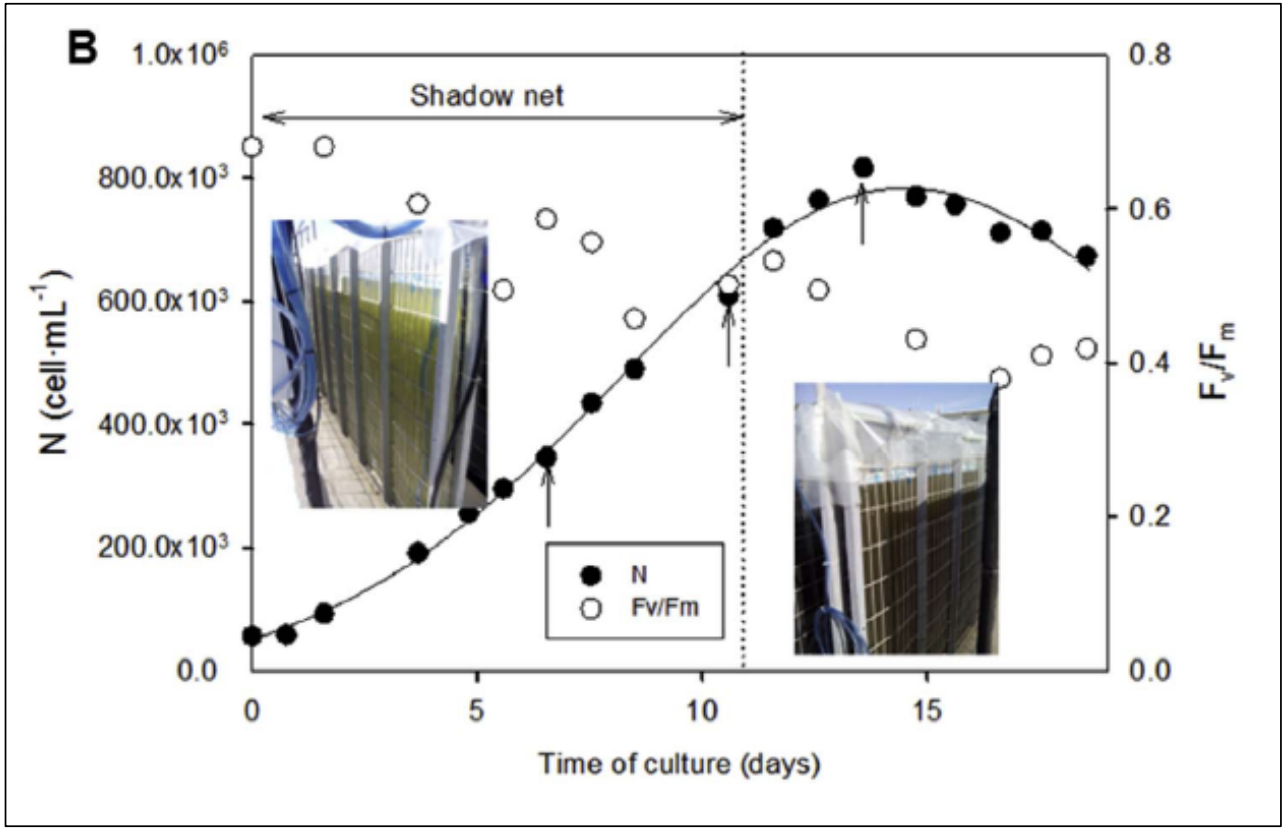
Microalgae-based bioprocesses and bioproducts

Photobioreactor design

Computational Fluid Dynamics (CFD) ***K. veneficum***







López-Rosales et al. (2018). Biores. Techn. 253, 94-104

Microalgae-based bioprocesses and bioproducts

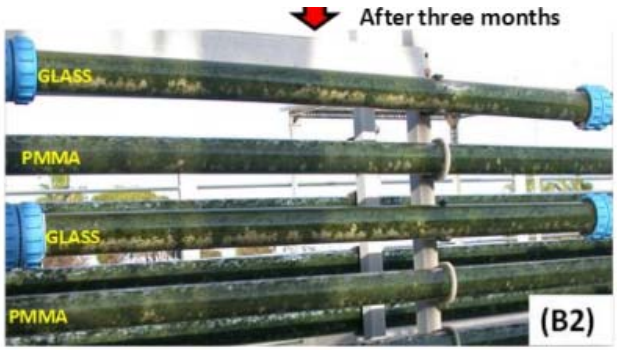
Photobioreactor design

ENHANCE MICROALGAE

Interreg Atlantic Area European Regional Development Fund EUROPEAN UNION

Antifouling surfaces

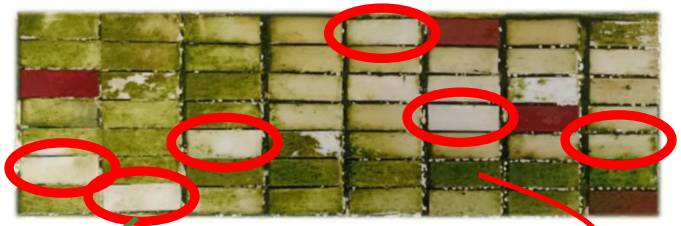
The aim of this project is to **develop** a new **transparent** and **non-toxic** surface to avoid microalgae **cell adhesion** to build an **efficient closed-PBR**



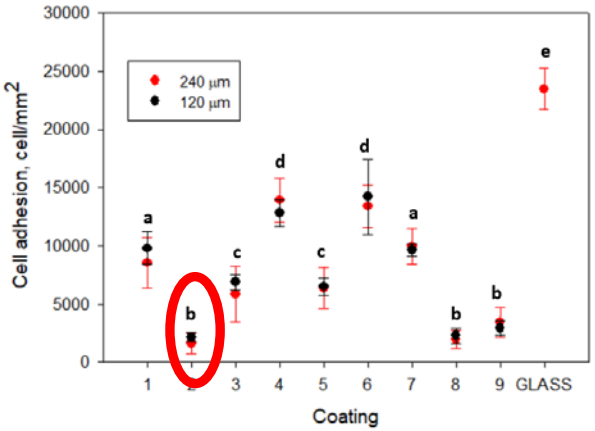
<u>Biofouling consequences</u>:

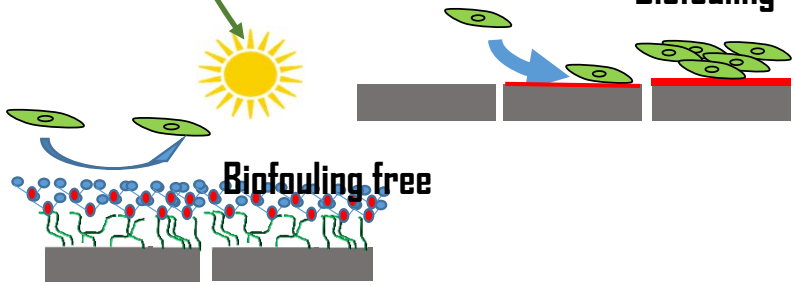
- Decrease solar radiation
- Negatively affect culture quality
- Less production
- Increase process cost

Day 0



8 months

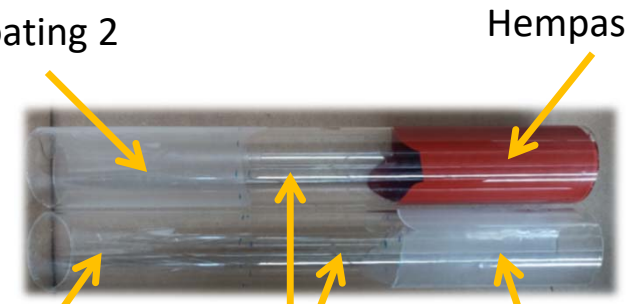


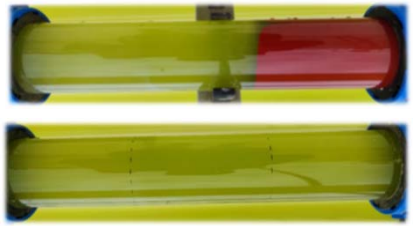


Microalgae-based bioprocesses and bioproducts

Photobioreactor design

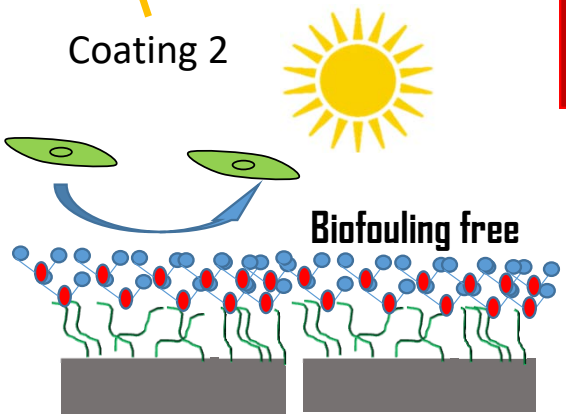
Antifouling surfaces





Day 0

Day 30



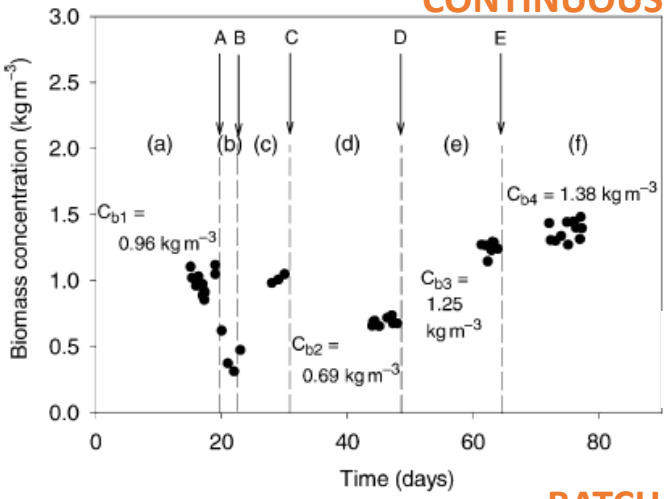
Testing at
Pilot Scale

Microalgae-based bioprocesses and bioproducts

Operational mode

Yield-based selection of operational mode

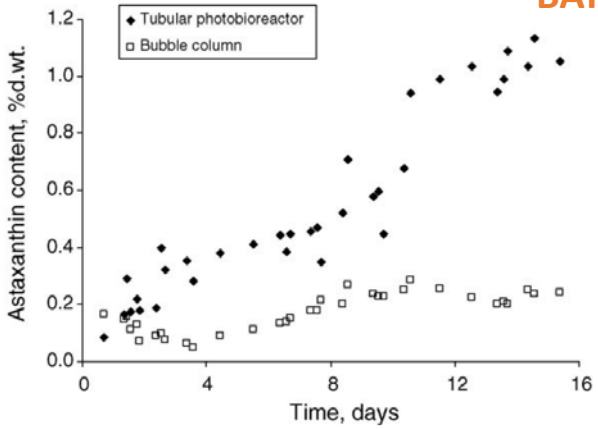
CONTINUOUS



Sánchez-Mirón et al. (2003). Biochem. Eng. J. 16, 287-297

P. tricornutum for PUFAs

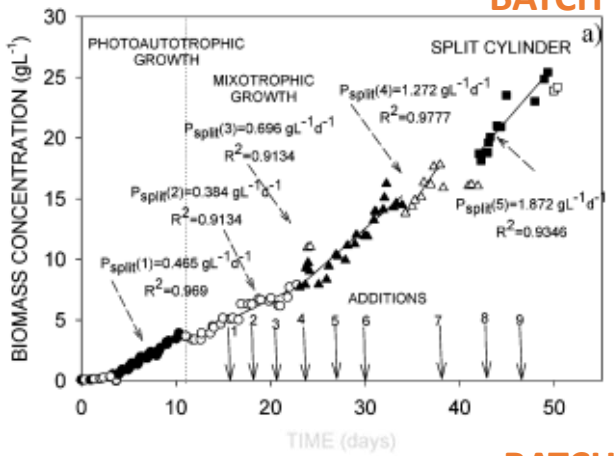
BATCH



García-Malea et al. (2006). J. Technol. 123, 329-342

H. pluvialis for astaxanthin photoautotrophically

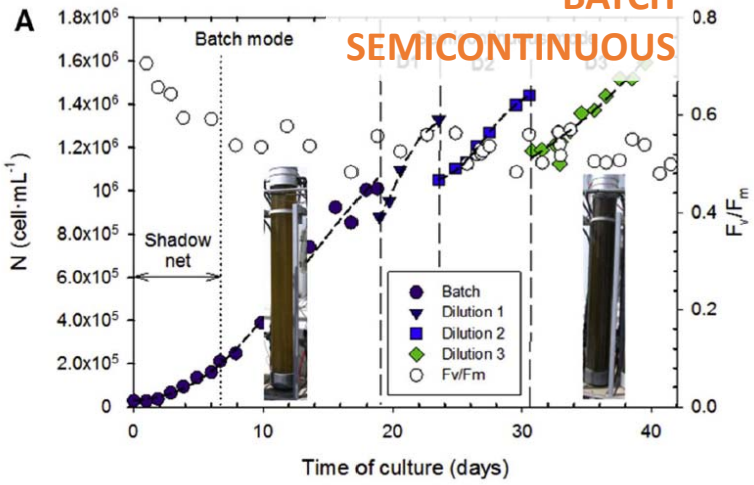
BATCH



Fernández Sevilla et al. (2004). Biotechnol. Prog. 2004, 20, 728−736

P. tricornutum for PUFAs and pigments mixotrophically

BATCH
SEMICONTINUOUS



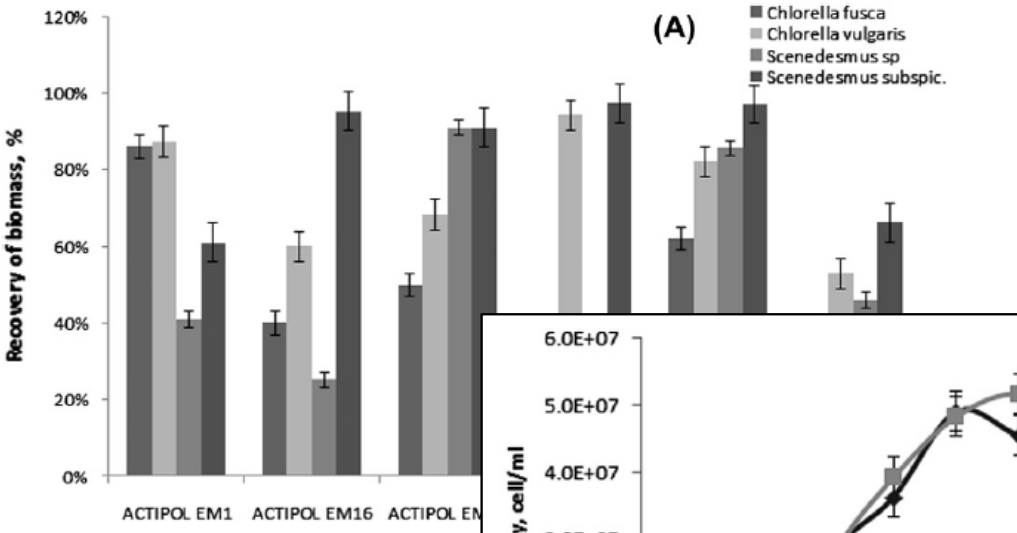
López-Rosales et al. (2018). Biores. Techn. 253, 94-104

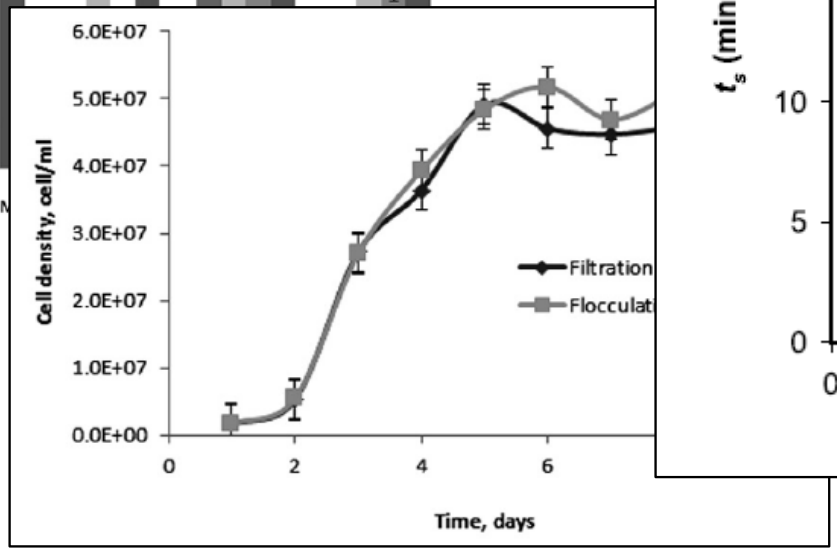
K. veneficum for karlotoxins

Microalgae-based bioprocesses and bioproducts

Harvesting

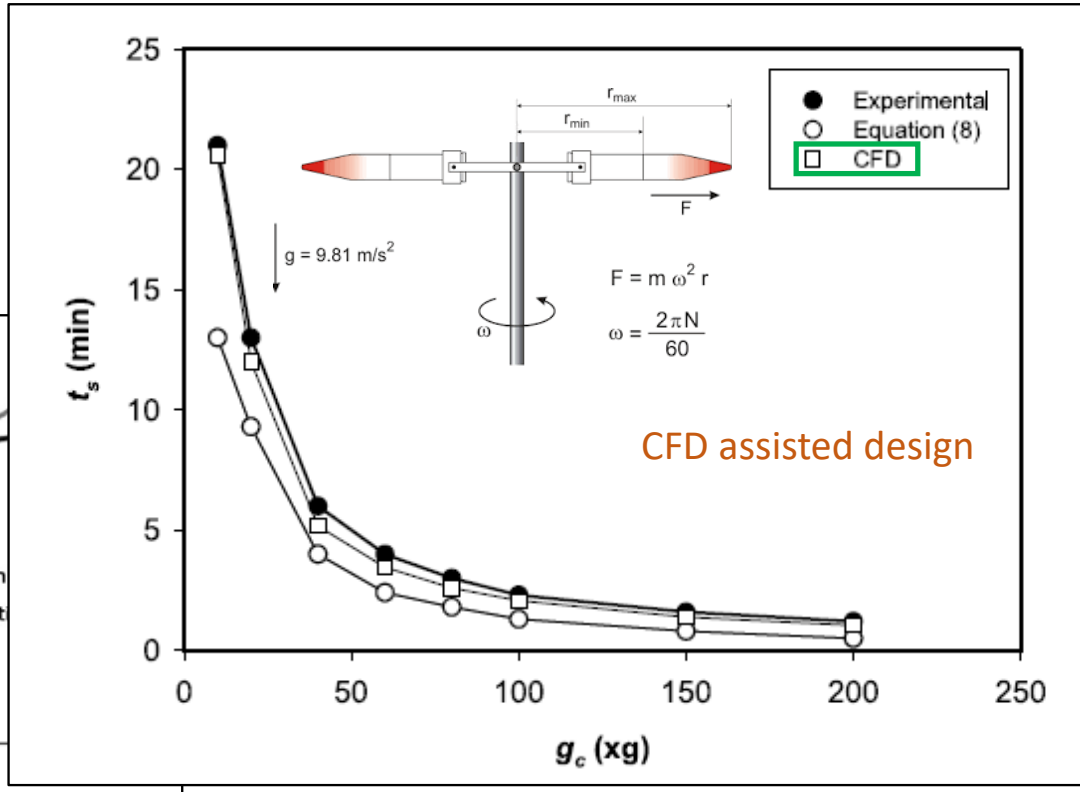
Scalable harvesting methods





The cell-free supernatant was not toxic for a subsequent reuse.

Granados et al. (2012). Biores. Techn. 118, 102-110



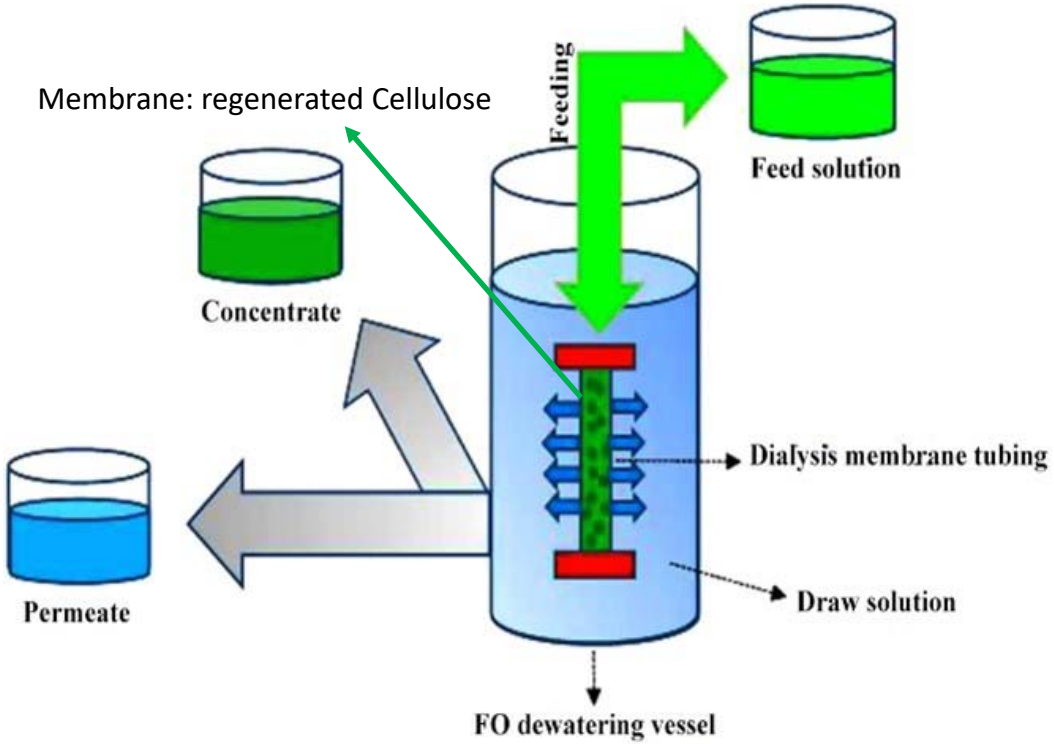
Molina-Miras et al. (2019). Algal Res. 44, 101677

Microalgae-based bioprocesses and bioproducts

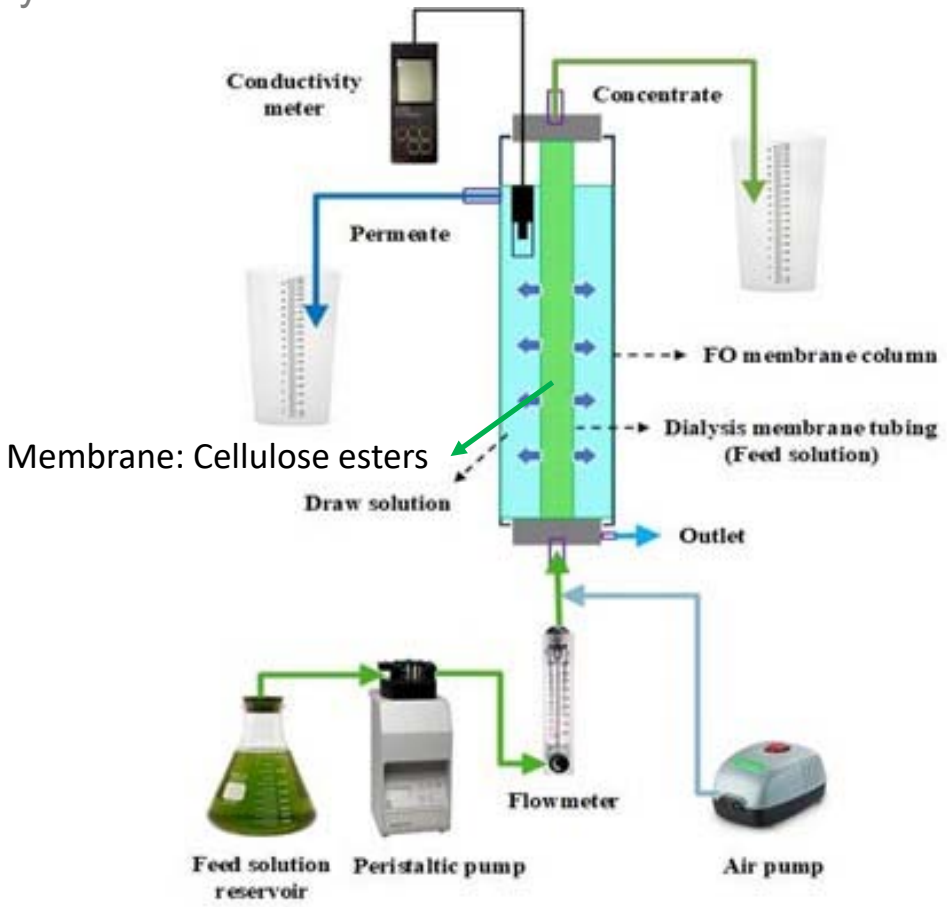
Harvesting

Osmotic dehydrator

Patent: P201400232



Concentration factor = 3



Concentration factor = 3

Microalgae-based bioprocesses and bioproducts

Extraction

Direct enzymatic transesterification of wet biomass

N. gaditana

High pressure homogenization (140 MPa), facilitate access

Solvents: 1-butanol, MeOH

Transesterification using as catalyst the lipase **Novozym 435**

FAMEs, solvents, biomass residues, lipase, water

Two-step Liquid-Liquid Extraction (Hexane)

Residue (Hidroalcoholic phase)

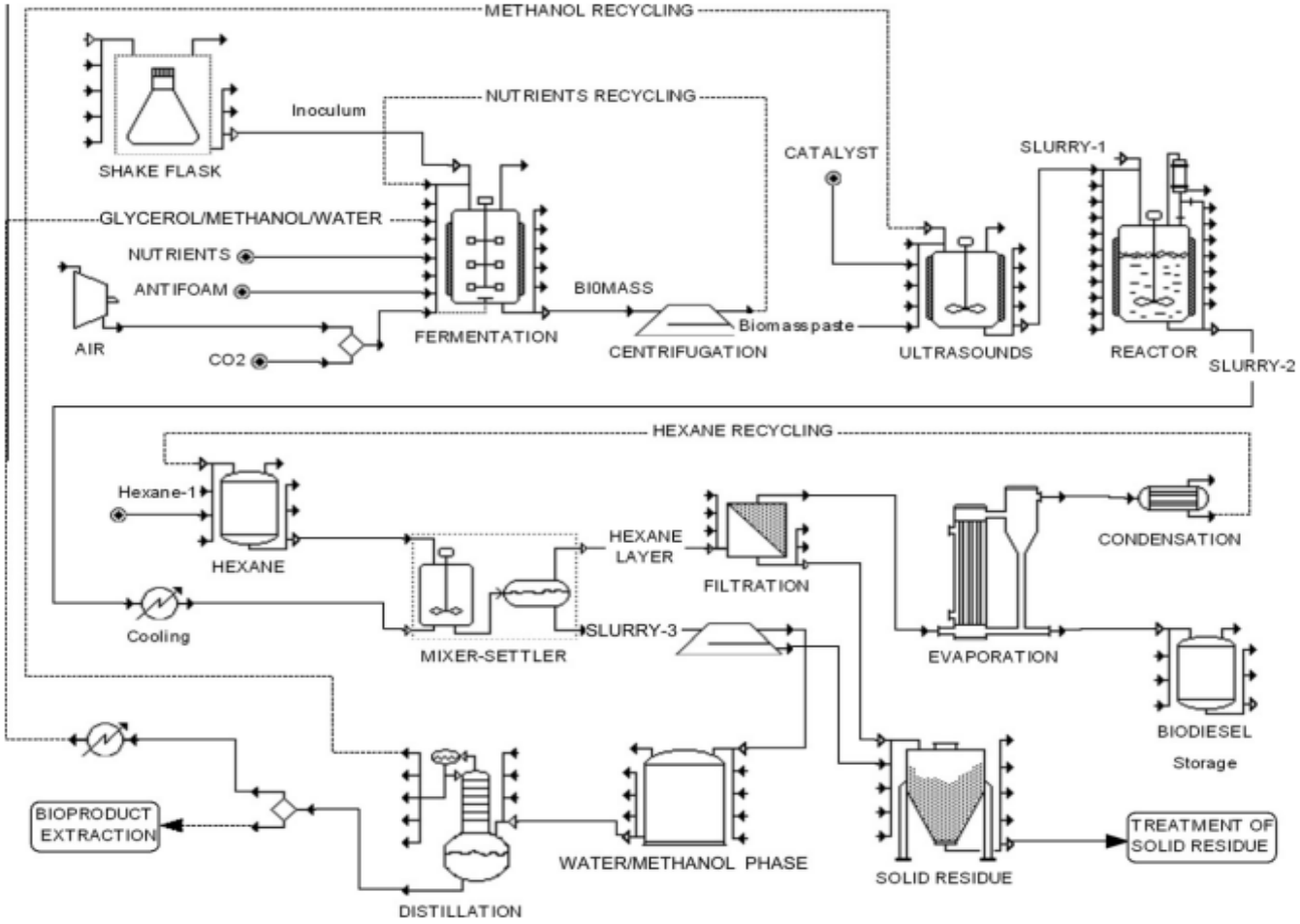
Hexanic phase containing FAMEs (99,5% yield)

Navarro López et al. (2016). Biomass Bioenergy 93, 6-12

Optimization of the production of FAMEs (biodiesel)

Microalgae-based bioprocesses and bioproducts

Extraction



Optimized process for *C. protothecoides* heterotrophic biomass

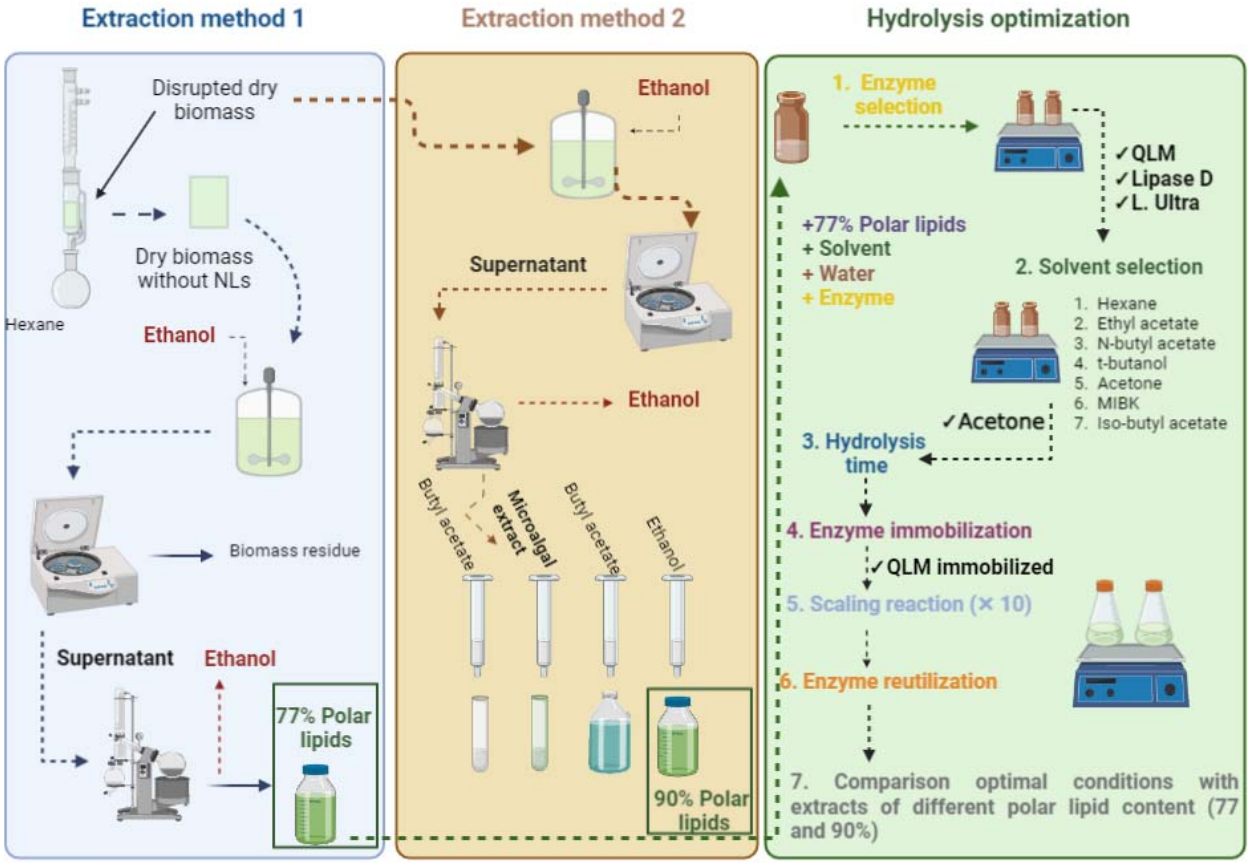
Fatty acids	%
C16:0	9.0
C16:1	1.4
C18:0	4.5
C18:1	66.1
C18:2	11.9
C18:3	1.0

Microalgae-based bioprocesses and bioproducts
Extraction

EPA in polar lipids

Process comprising:

- Simultaneous extraction and fractionation from *Nannochloropsis* sp.
- Silica-gel chromatography using non-toxic solvents
- Lipase catalysed hydrolysis



Jiménez et al. (2020) J Appl. Phycol. 32, 1117-28; Macías et al. (2022). Biomass Conv. Bioref. (2022) doi.org/10.1007/s13399-022-02520-2

Microalgae-based bioprocesses and bioproducts
Extraction

Biostimulants and fitosanitaries

PLANT BIOSTIMULANT

Scenedesmus sp.

Wet biomass from *Scenedesmus* sp. grown in pig manure

High pressure homogenization (HPH)

Incubation (30 ºC 2 h, 300 rpm)

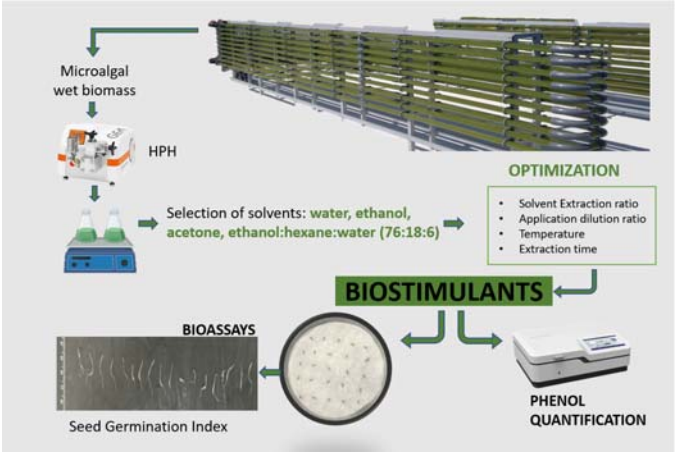
Enzymatic hydrolysis

Centrifugation 12000 rpm, 5 min

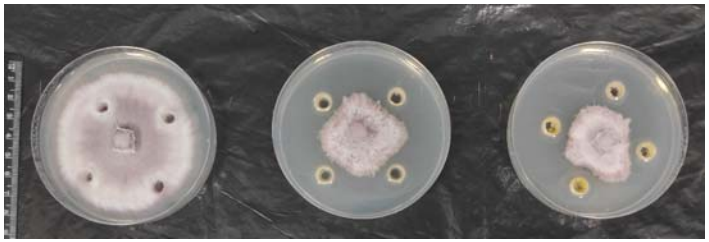
BIOFERTILIZERS

Pellet

BIOSTIMULANTS



200 bar Germination Index >10%



(19) OFICINA ESPAÑOLA DE PATENTES Y MARCAS

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(11) Número de publicación: **2 908 029**

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(51) Int. Cl.:

A01N 65/03 (2009.01)

A01N 25/02 (2006.01)

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(12) SOLICITUD DE PATENTE A1

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(71) Solicitantes:
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CERÓN GARCÍA, Mª Del Carmen;
ROJAS CRESPO, Elisa;
GARCÍA LÓPEZ, Juan José y
POZO DANGRA, Joaquín

(74) Agente/Representante:
GONZÁLEZ PECES, Gustavo Adolfo

(54) Título: **Proceso para la obtención, a nivel industrial, de un extracto concentrado en compuestos bioplaguicidas a partir de microalgas y/o cianobacterias, extracto así obtenido y uso del mismo**

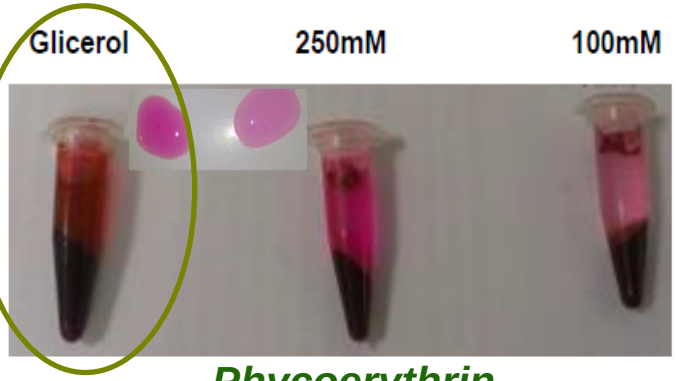
Navarro López et al. (2020) Algal Res. 52, 102123

Microalgae-based bioprocesses and bioproducts

Extraction

Phycobiliproteins using glycerol

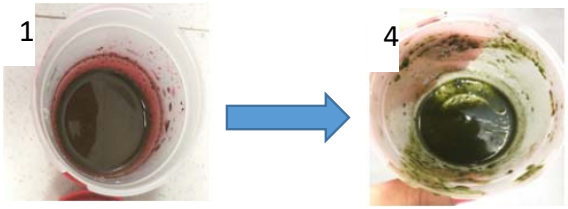
Comparison of glycerol-acetate buffer from fresh biomass



Phycoerythrin
P. purpureum

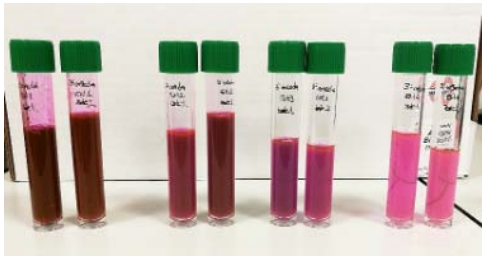
Improved extraction from fresh biomass (5 times better)

Comparison glycerol + successive acetate buffer from fresh or frozen biomass



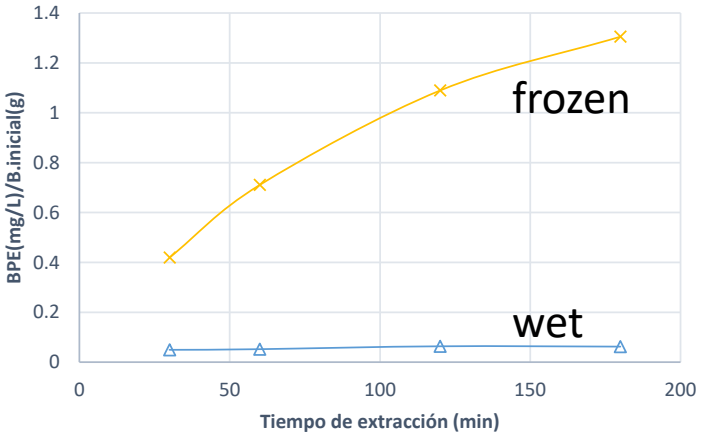
Initial extractant mixture

residual biomass



1 2 3 4

Number of extractions



Even better from frozen biomass

Patent: P201600100

Microalgae-based bioprocesses and bioproducts

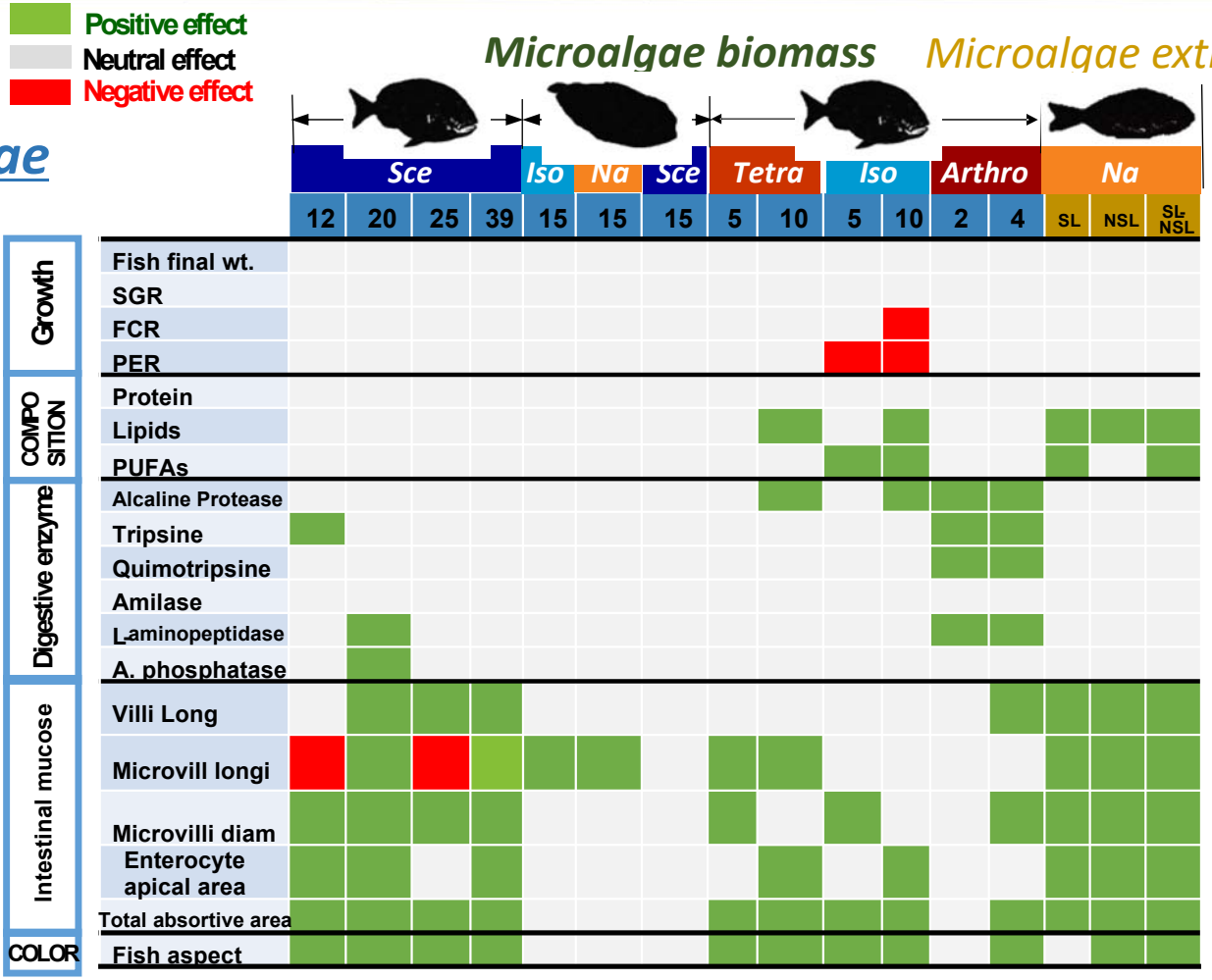
Extraction

Microalgae

Experimental diets

- Control diet (CT)
- Microalgal diets
- Carotenoid diets
- PUFAs diets
- PUFAs+Carot diets

FISH DIETS



- ✓ Positive effect on health state of the gut mucose favouring the absorption of the nutrients.
- ✓ Improvement of fish meat quality (EPA).

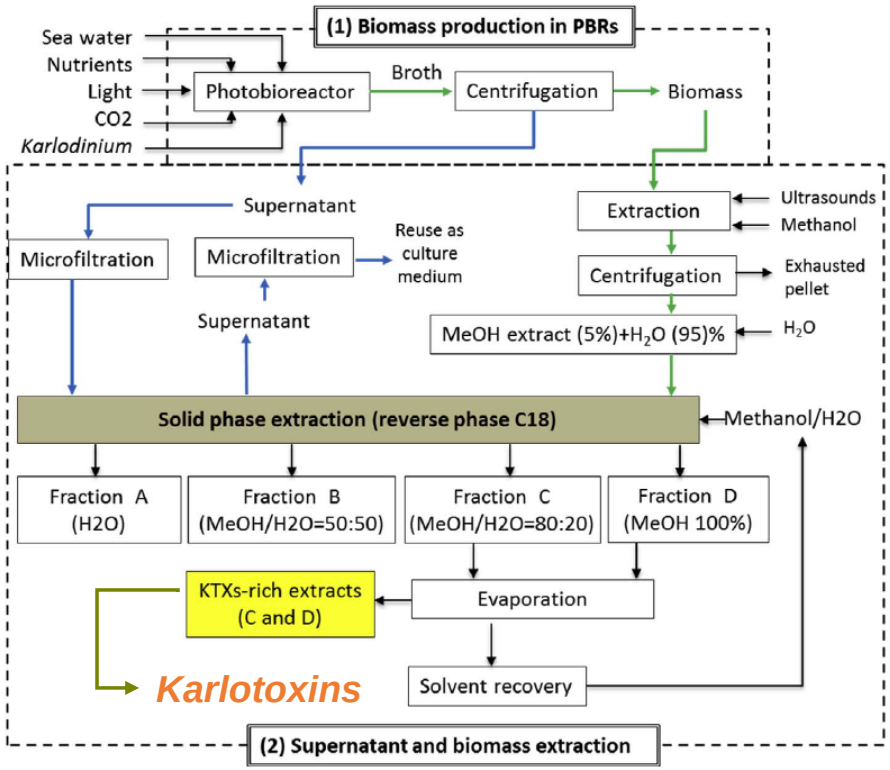
Vizcaíno et al. (2014). Aquaculture. 431, 34-43; Vizcaíno et al. (2016). J. Appl. Phycol. 28(5), 2843-2855; Vizcaíno et al. (2018). Fish Physiol. Biochem. 44(2), 661-667; Sales et al. (2021). Algal Res. 53, 102162

Microalgae-based bioprocesses and bioproducts

Purification

Scalable purification processes of bioactives and high value compounds

K. veneficum



S. almeriensis

OFICINA ESPAÑOLA DE PATENTES Y MARCAS
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C09B 67/54 (2006.01)
B01D 11/02 (2006.01)
B01D 11/04 (2006.01)

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(43) Fecha de publicación del folleto de la solicitud: **14.05.2009**

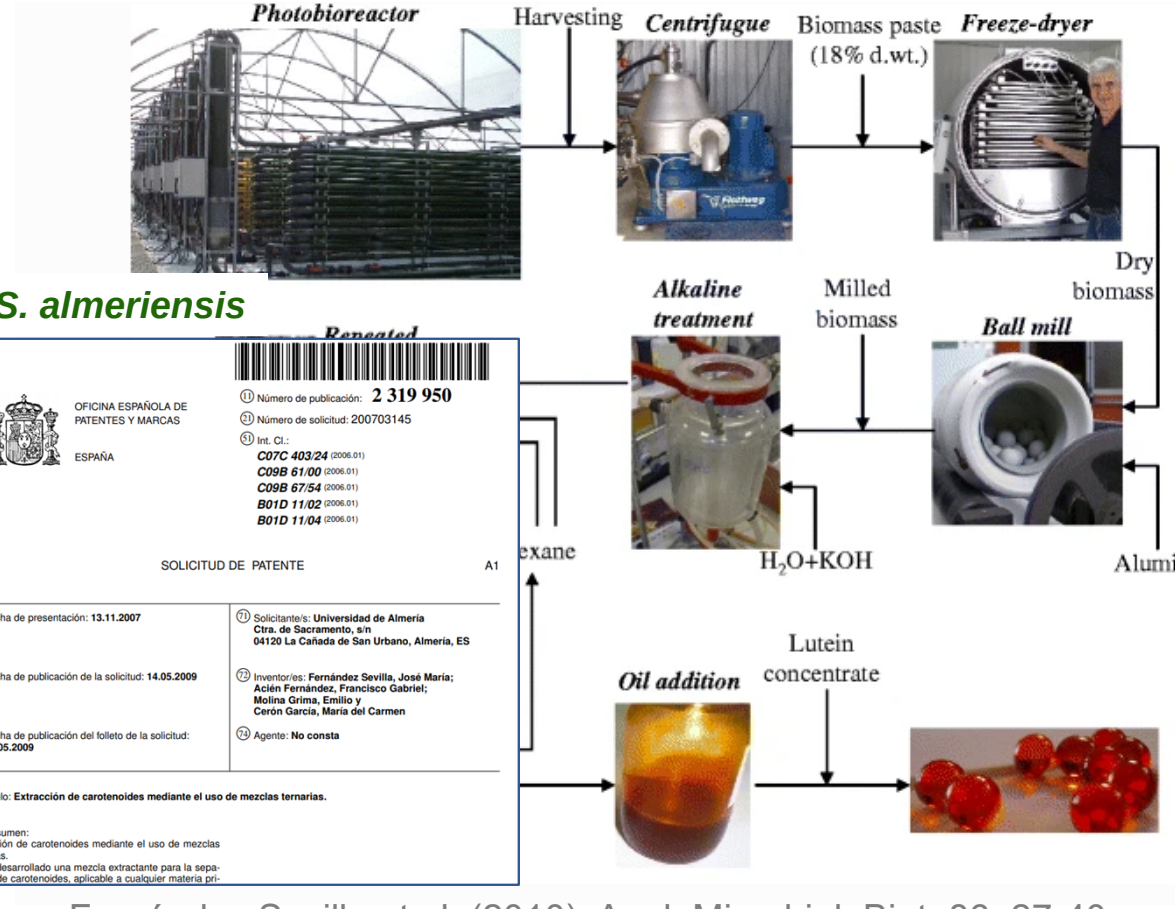
(71) Solicitante/s: **Universidad de Almería**
Ctra. de Sacramento, s/n
04120 La Cañada de San Urbano, Almería, ES

(72) Inventor/es: **Fernández Sevilla, José María; Acién Fernández, Francisco Gabriel; Molina Grima, Emilio y Cerón García, María del Carmen**

(74) Agente: **No consta**

(54) Título: **Extracción de carotenoides mediante el uso de mezclas ternarias.**

(57) Resumen:
Extracción de carotenoides mediante el uso de mezclas ternarias.
Se ha desarrollado una mezcla extractante para la separación de carotenoides, aplicable a cualquier materia pri-



López-Rosales et al. (2018). Biores. Techn. 253, 94-104

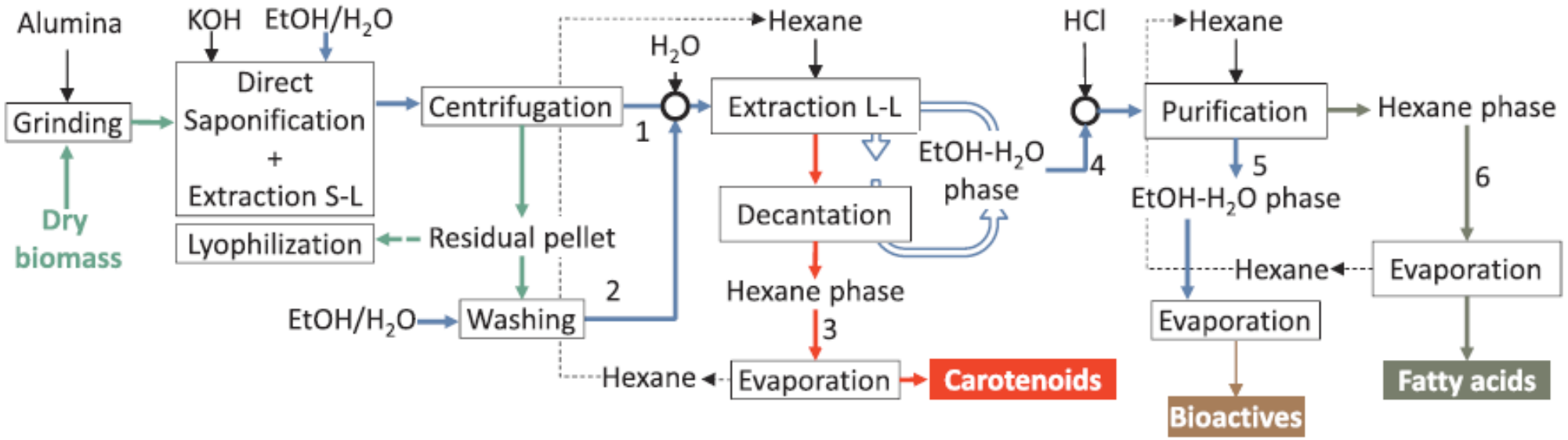
Fernández Sevilla et al. (2010). Appl. Microbiol. Biot. 86, 27-40

Microalgae-based bioprocesses and bioproducts

Purification

A. carterae

Purification of the main bioproducts



López-Rodríguez et al. (2019). Biores. Techn. 282, 370-377

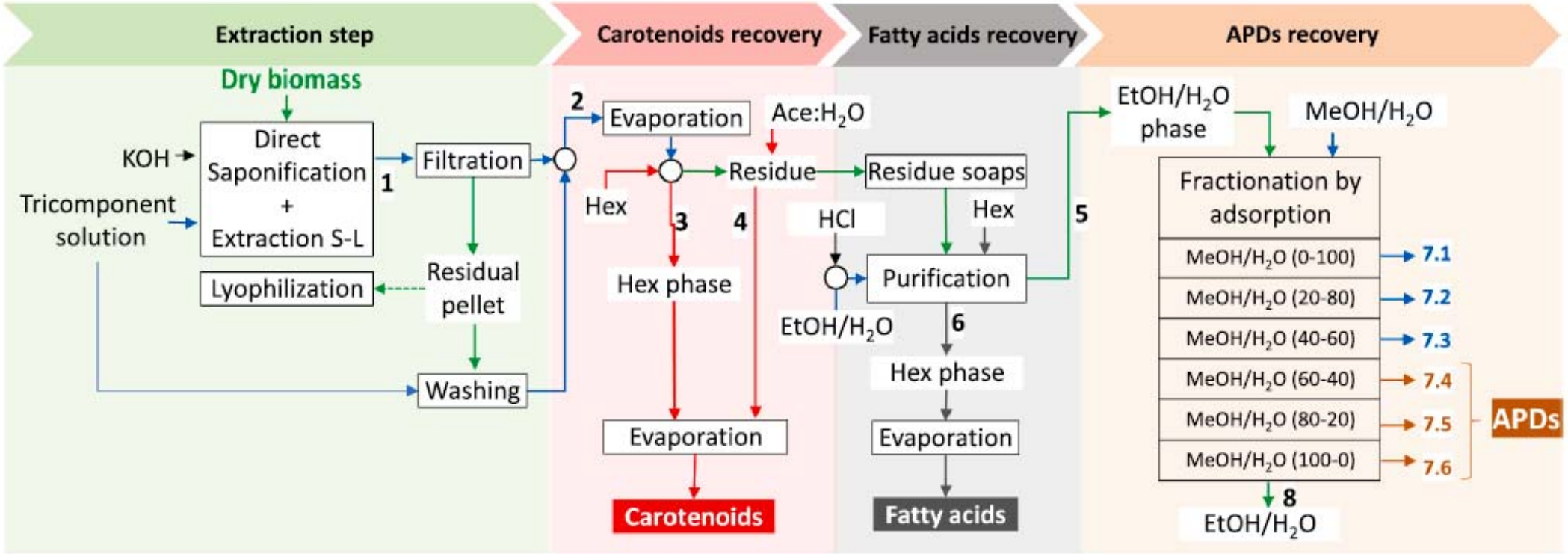
López-Rodríguez et al. (2021). Biores. Techn. 342, 125922
López-Rodríguez et al. (2022). Toxins, 14(9), 593

Microalgae-based bioprocesses and bioproducts

Purification

A. carterae

Purification of the main bioproducts



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López-Rodríguez et al. (2021). Biores. Techn. 342, 125922
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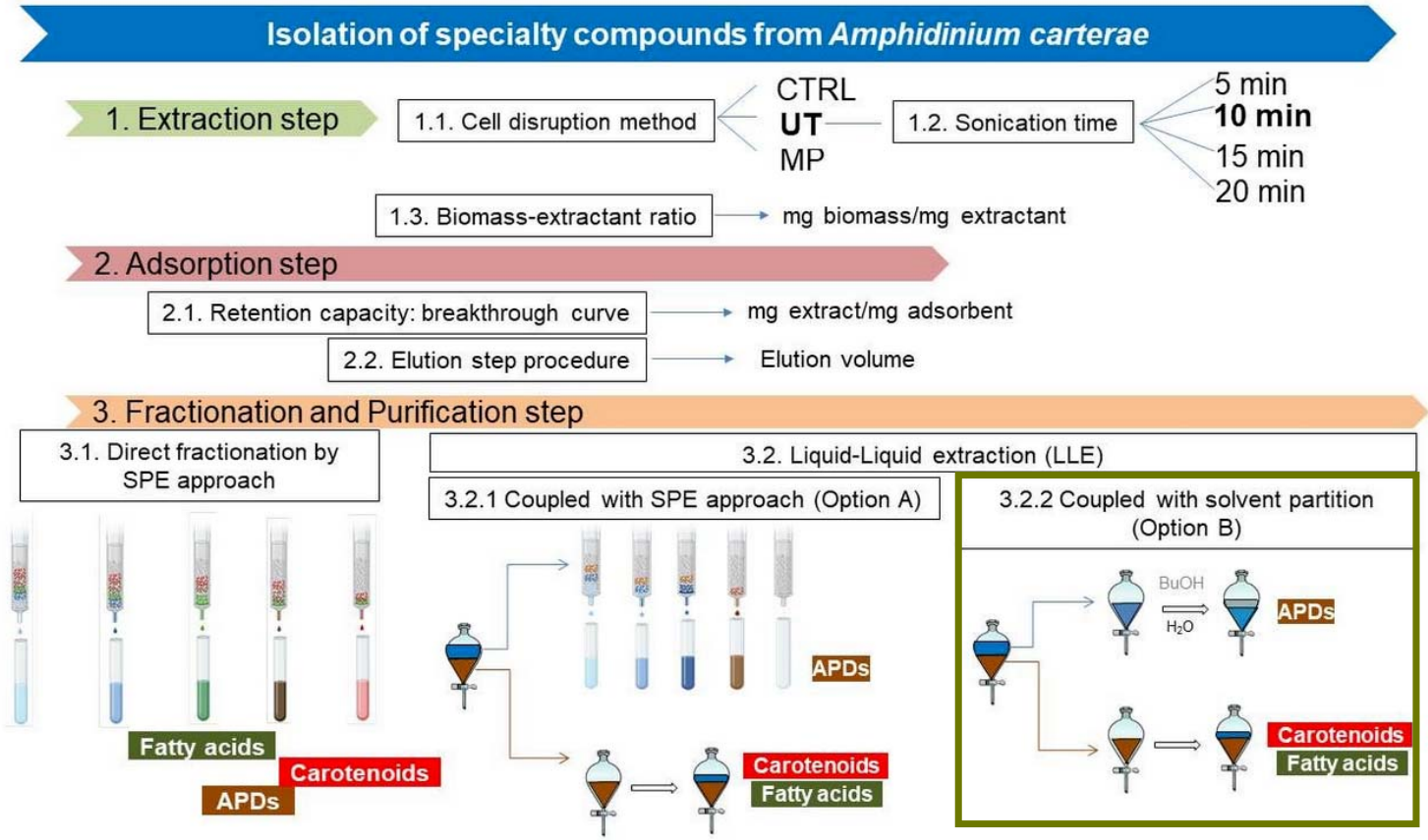
Microalgae-based bioprocesses and bioproducts
Purification

A. carterae

Purification of the main bioproducts



López-Rodríguez et al. (2019). Biores. Techn. 282, 370-377

APDs rich extract were purified at a 70% concentration (w/w)

Solvent partition in an only process obtained the best results for the three families of compounds, carotenoids, fatty acids and APDs.

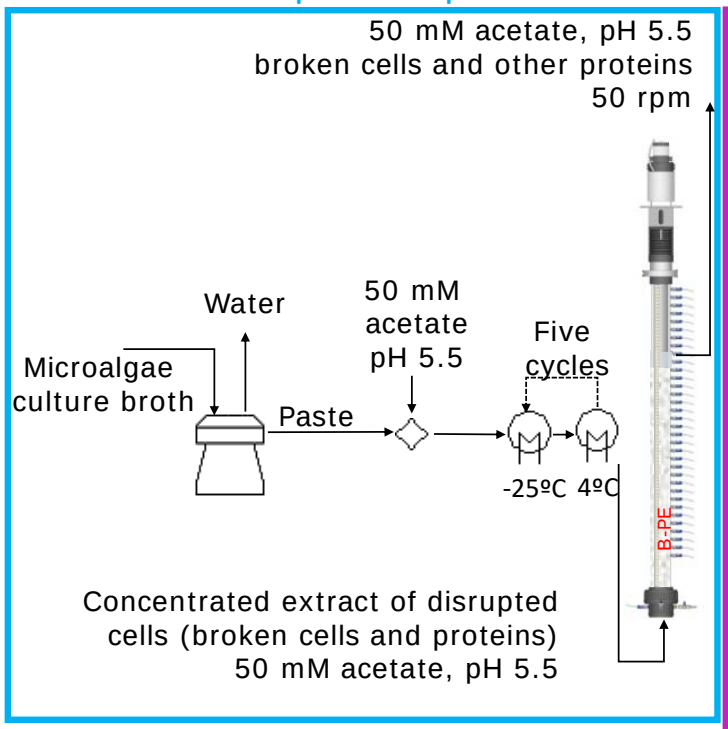
López-Rodríguez et al. (2021). Biores. Techn. 342, 125922
López-Rodríguez et al. (2022). Toxins, 14(9), 593

Microalgae-based bioprocesses and bioproducts

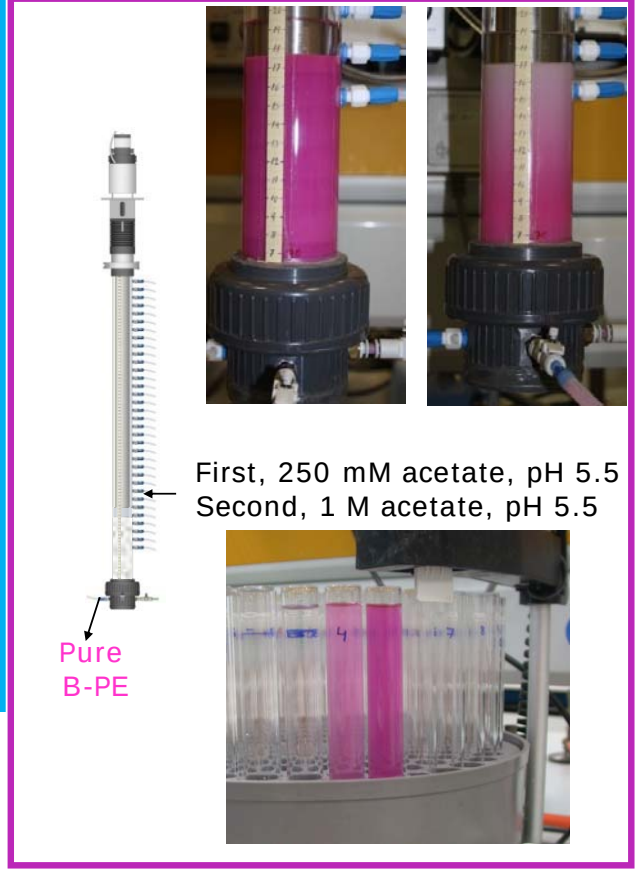
Purification

Vortex Flow Reactor: expanded bed chromatography

Adsorption step



Elution step



Purification of

β-Phycoerythrin

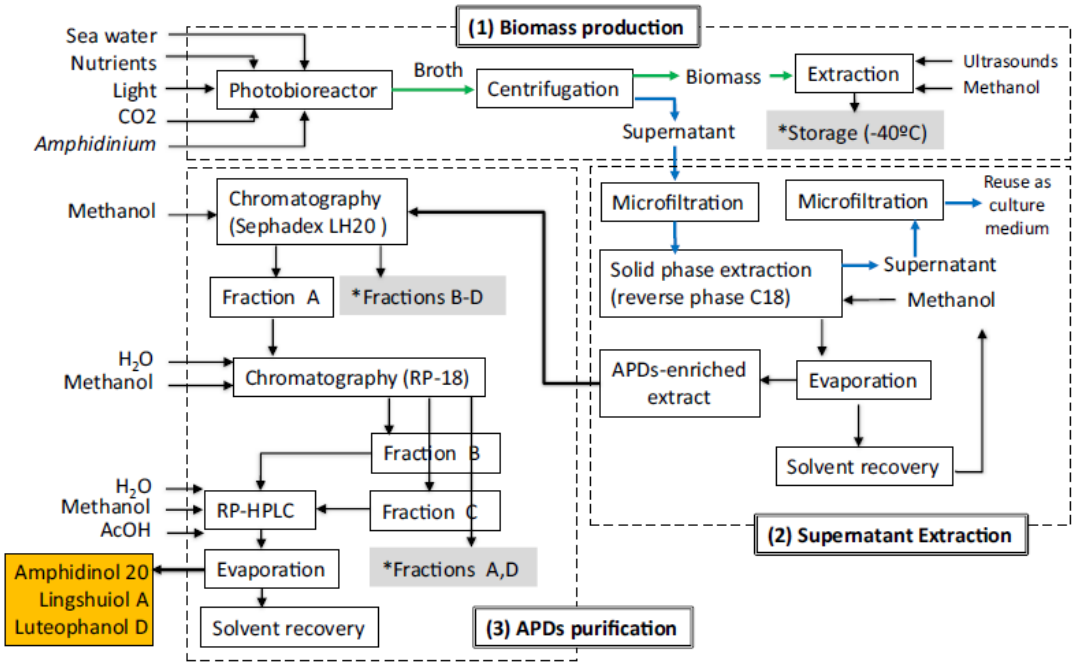
Expanded bed vortex flow reactor unit operation in the downstream process eliminates the previous filtration step

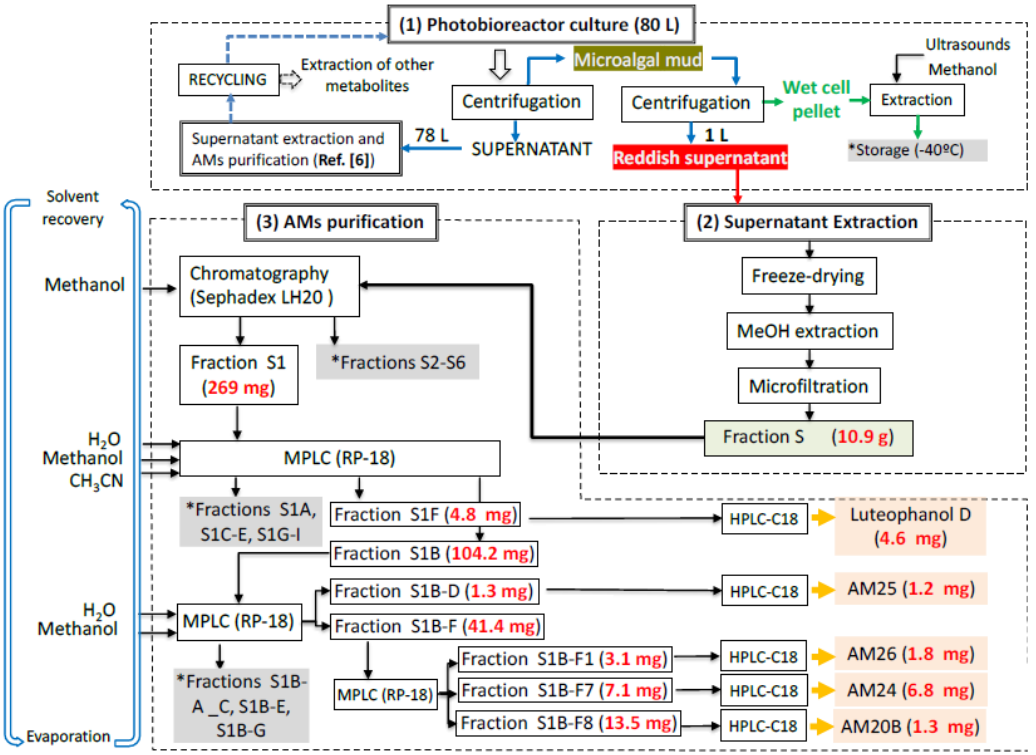
Microalgae-based bioprocesses and bioproducts

Identification

Identification of different bioactives

A. carterae





Molina-Miras et al. (2018). Algal Res. 31, 87-98

Morales-Amador et al. (2021). Mar. Drugs 19, 432


Thank you!
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ENHANCE
MICROALGAE
Interreg
Atlantic Area
European Regional Development Fund
EUROPEAN UNION