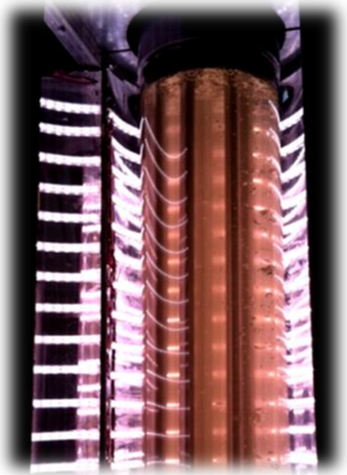
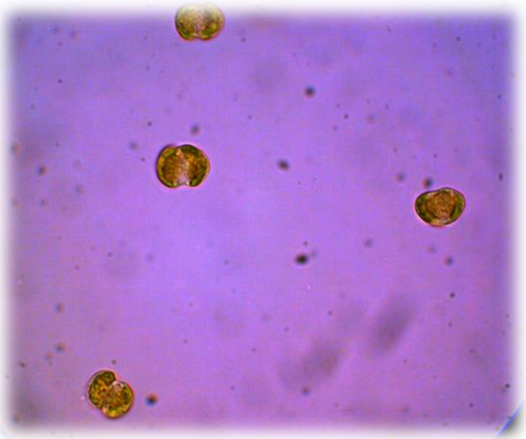
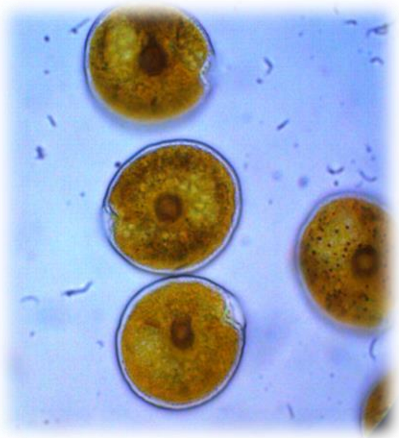
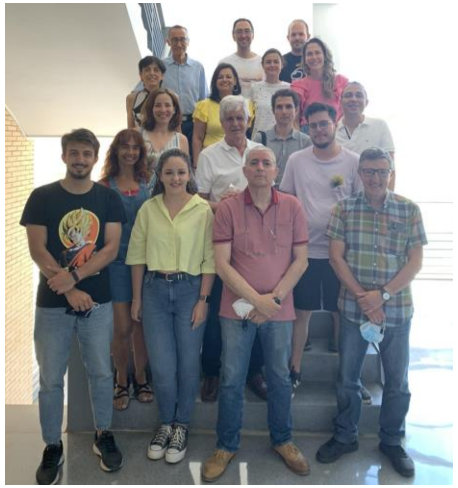
Marine Microalgae Biotechnology
(Biotechnological potential of marine dinoflagellates)

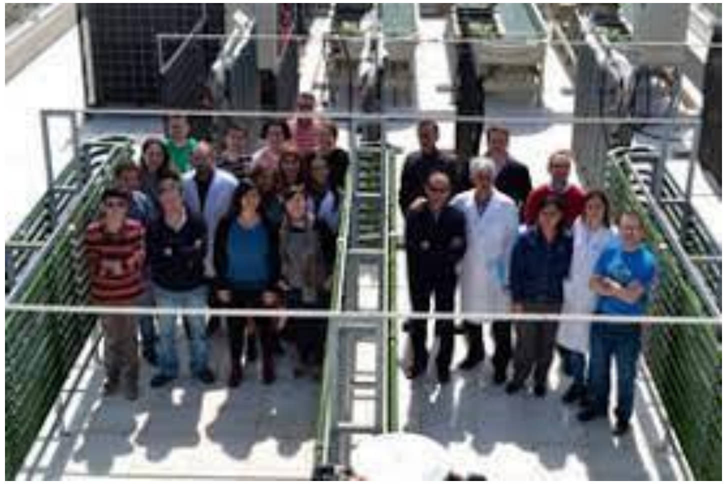


Lorenzo López Rosales (Universidad de Almería)

Marine Microalgae Biotechnology Research Group (BIO173)





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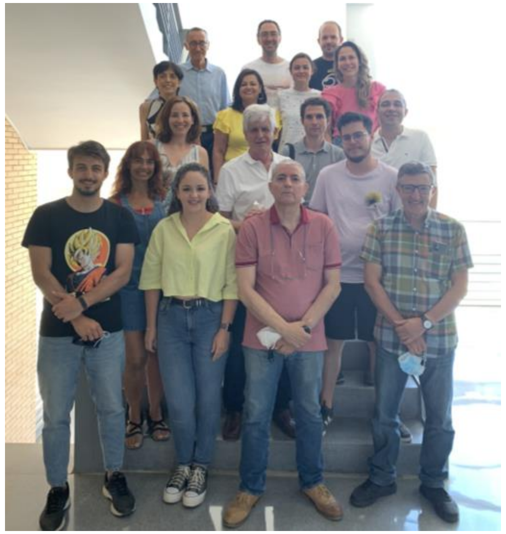
- Chemical Engineering Department
- Engineering treatment of bioprocesses

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

Emilio Molina Grima

Asterio Sánchez Mirón

Francisco García Camacho

- L … facilities … croalgal biomas
  c … osed ph
- T … harvesti … and downstrea
- E … fractions … ication of biom
  r

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1. Overview

OUTLINE

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1.-Camacho, F. G., Rodríguez, J. G., Mirón, A. S., García, M. C., Belarbi, E. H., Chisti, Y., & Grima, E. M. (2007). Biotechnological significance of toxic marine dinoflagellates. Biotechnology advances, 25(2), 176-194.

2.-Gallardo-Rodríguez, J., Sánchez-Mirón, A., García-Camacho, F., **López-Rosales, L**., Chisti, Y., & Molina-Grima, E. (2012). Bioactives from microalgal dinoflagellates. Biotechnology advances, 30(6), 1673-1684.

3.-García-Camacho, F., Sánchez-Mirón, A., Gallardo-Rodríguez, J., **López-Rosales, L.**, Chisti, Y., & Molina-Grima, E. (2014). Culture of microalgal dinoflagellates. In Seafood and Freshwater Toxins: Pharmacology, Physiology, and Detection (pp. 551-566). CRC Press Boca Raton.

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Marine Dinoflagellates

OUTLINE

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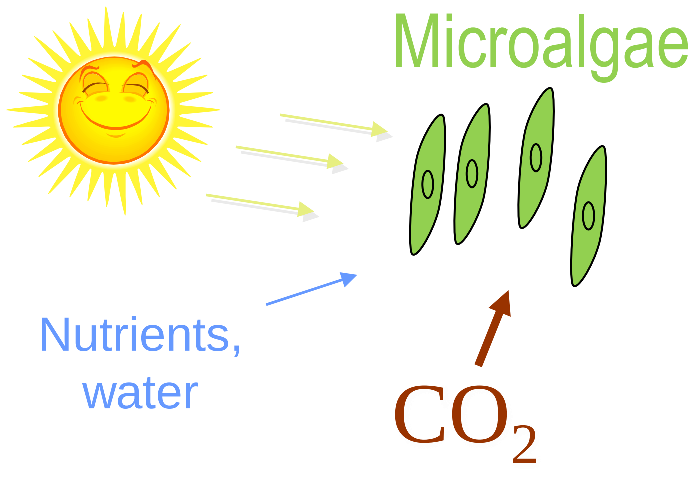
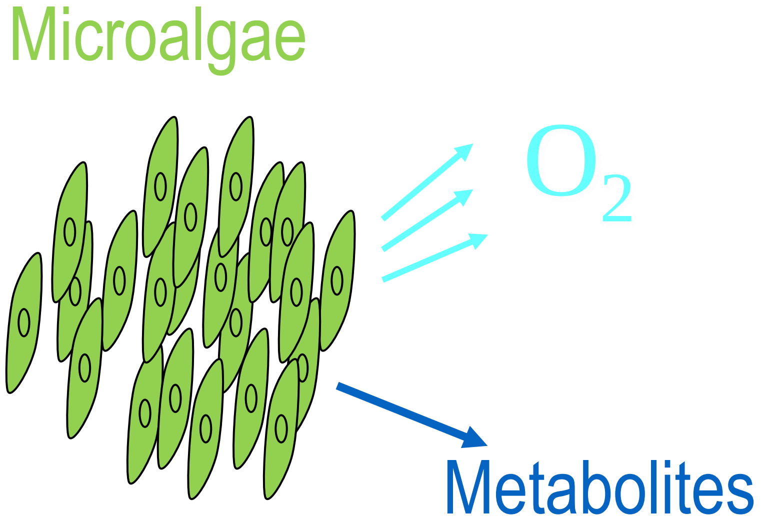
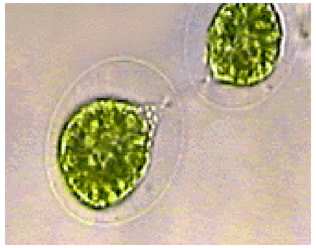
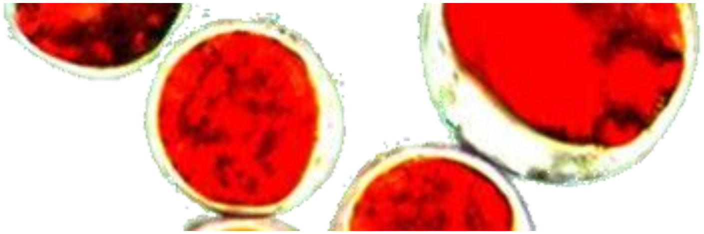
Photoautotrophic unicellular microorganisms

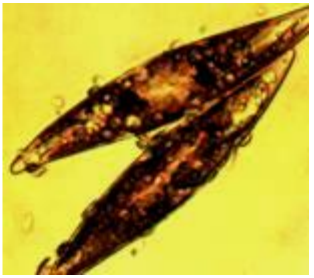




- High duplication speed

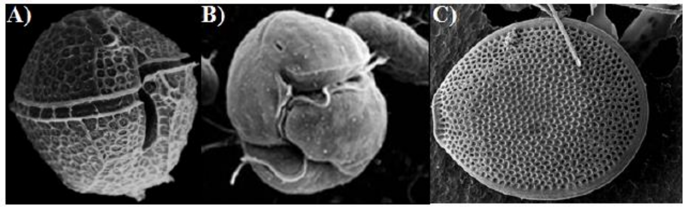






Marine Dinoflagellates

➢ Microalgae producing bioactive substances



Biocompounds

FOOD SECTOR

Production of certified Reference standards for seafood safety testing

THERAPEUTIC DRUGS

Antitumorals (i.e. Amphidinolides, symbioramide, Goniodomin-A, Yessotoxins, Pectenotoxins, etc.)
Antifungals (i.e. gambieric acids,amphidinols)
Antibiotics (i.e. amphidinolides, goniodomin)
Anesthetics (i.e. neosaxitoxins)
Prevention and treatment of osteoporosis (i.e. symbioramide)
Treatment of heart diseases (i.e. karlotoxins)

OUTLINE

1. **Overview**
2. Objectives
3. Strategies

3.1 Improvement of culture media

3.2 Modelling the growth of dinoflagellates with ANNS

3.3 Optimization of culture conditions

3.4 Recycling recourses

3.5 Scale-up of the bioprocess

4. Conclusions

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Prices of commercial biotoxins from dinoflagellates

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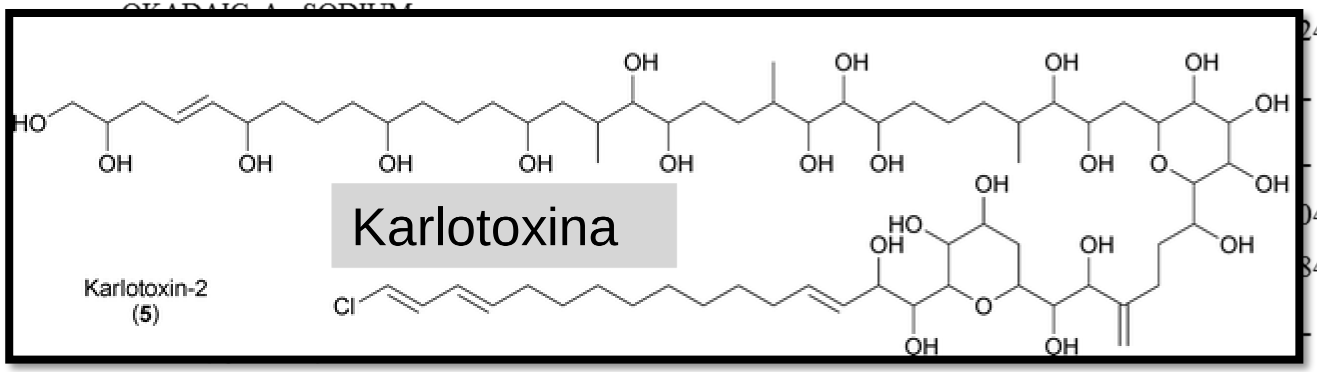
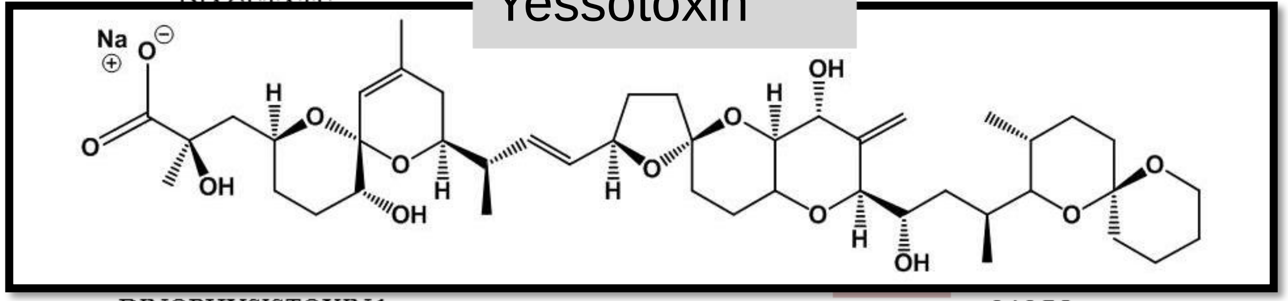
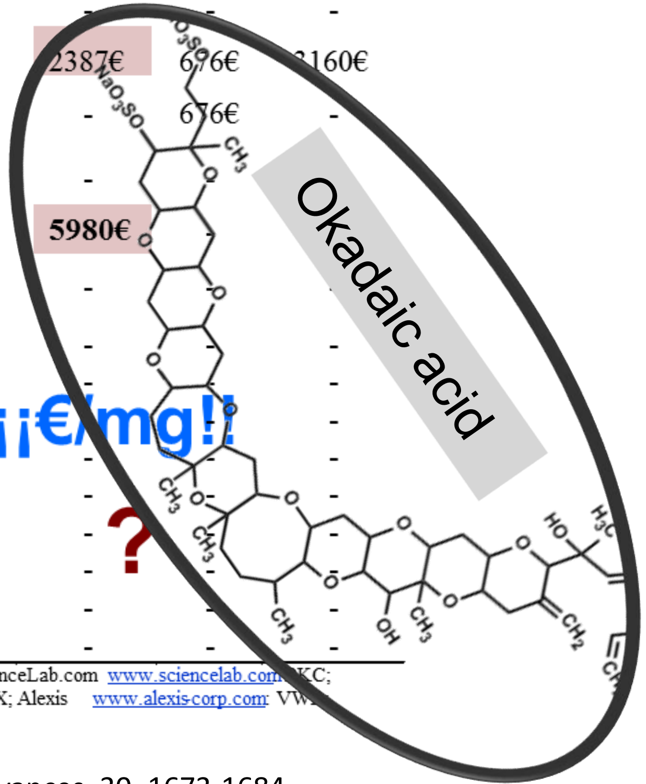
- High structural complexity (no chemical synthesis)
- Large scale cultivation

...om 2006 catalogues. The prices are ... methods can be find in the corresponding ... -producing species indicated by suppliers were *Prorocentrum concavum*, *Ptychodiscus* ... *iscus toxicus*, *Palythoa caribaeorum*, y *Protogonyalaux sp*

BIOTOXINAS	SGA	ALEX	LKT	VWR	WK*	LD	GEN	SL	PKC	TK
OKADAIC A. SODIUM ...	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	...24€	1849€	**676€**	-
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	-	3870€	676€	-
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	-	-	-	-
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	...04€	2387€	676€	...160€
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	...84€	-	676€	-
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	-	-	[illegible]	[illegible]
OKADAOL	5530€	-	-	3089€	-	4420€	-	**5980€**	[illegible]	[illegible]
SAXITOXIN, DIACETATE	1...	[illegible]	[illegible]	-	-	-	-	-	[illegible]	[illegible]
DINOPHYSISTOXIN-1	-	-	-	-	**2125€**	-	-	-	[illegible]	-
PECTENOTOXIN-6	-	-	-	-	**2125€**	-	-	-	[illegible]	-







¡¡€/mg!!

?

SGA; Sigma-Aldrich www.sigmaaldrich.com GEN; Gentaur Molecular Products www.gentaur.com LD; The lab Depot www.labdepotinc.com SL; ScienceLab.com www.sciencelab.com PKC; PKC pharmaceuticals Inc www.lclabs.com TK; Tocris www.tocris.com BIO; WK; Wako Pure Chemical Industries Ltd www.wako-chem.com ALEX; Alexis www.alexis-corp.com VW... VWR International www.vwrsp.com

(*) Los okadaicos de Wako proceden de la esponja marina *Halichondria okadai*

Gallardo-Rodríguez, J. et al. (2012). Bioactives from microalgal dinoflagellates. Biotechnology Advances. 30, 1673-1684.

Drawbacks in the culture of marine dinoflagellates

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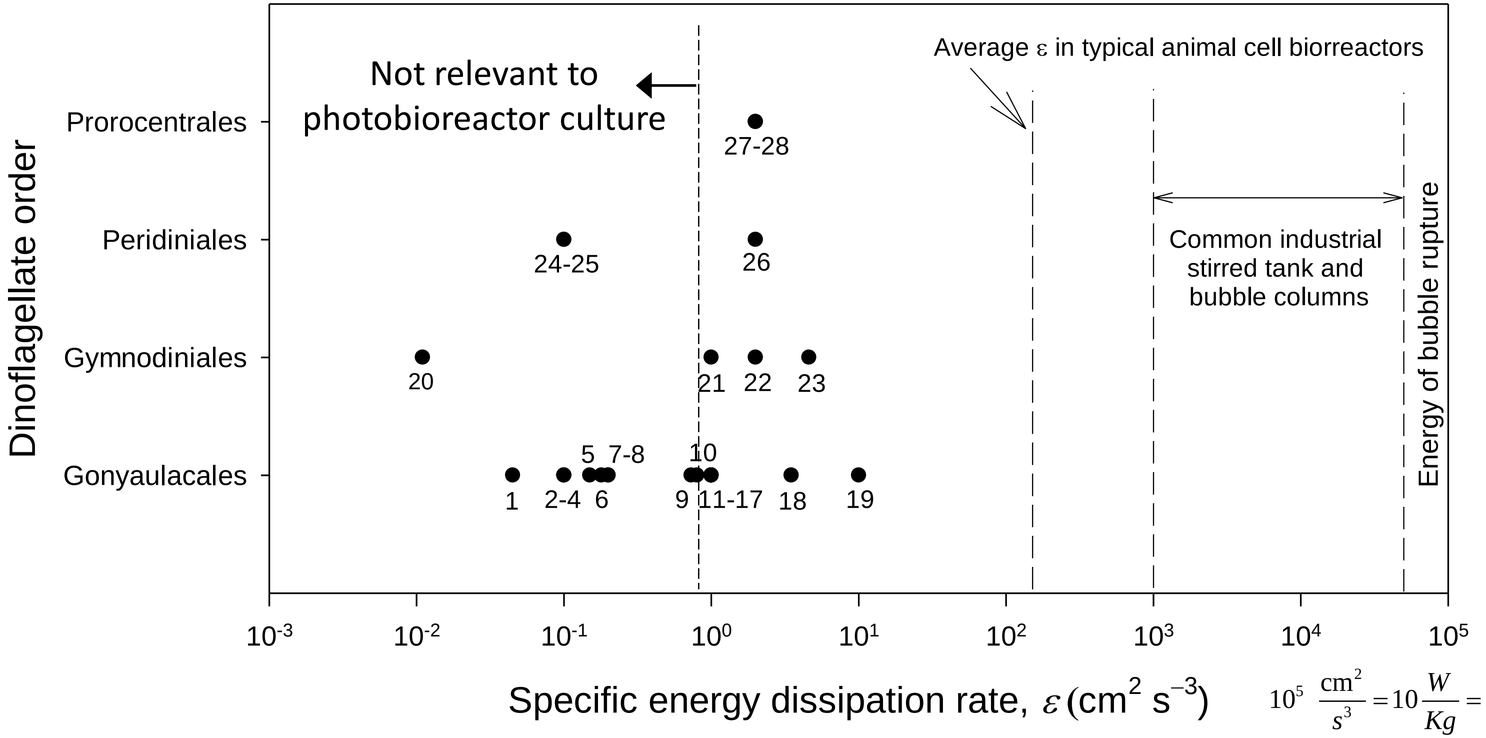
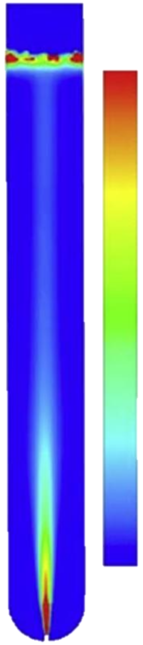
OUTLINE

- Very low bioactive titers (µg L^{-1})
- Low cell densities (mg L^{-1} or 10^{4-5} cell ml^{-1})
- **Shear-sensitive species**

- Low growth rates
- Complex metabolism
- Nutritional requirements



$$10^5\ \frac{cm^2}{s^3} = 10\frac{W}{Kg} = \left\{ \text{if } \rho_L = 1000\frac{Kg}{m^3} \right\} = 10^4\ \frac{W}{m^3}$$



Comparison of the damaging thresholds of specific energy dissipation rate reported for various orders of dinoflagellates and the average damage threshold reported for various kinds of animal and plant cells cultured in bioreactors.

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Amounts of product needed in a pharmaceutical application

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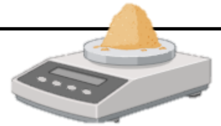
- Discovery of potential drug candidate structure(s) by screening compound/extract libraries.
- Production for preclinical phases



¡From several grams to hundreds of grams!

- Production for clinical phases

Multi-kilogram quantities

Major hurdle in the development of drugs from marine dinoflagellates

To ensure supply

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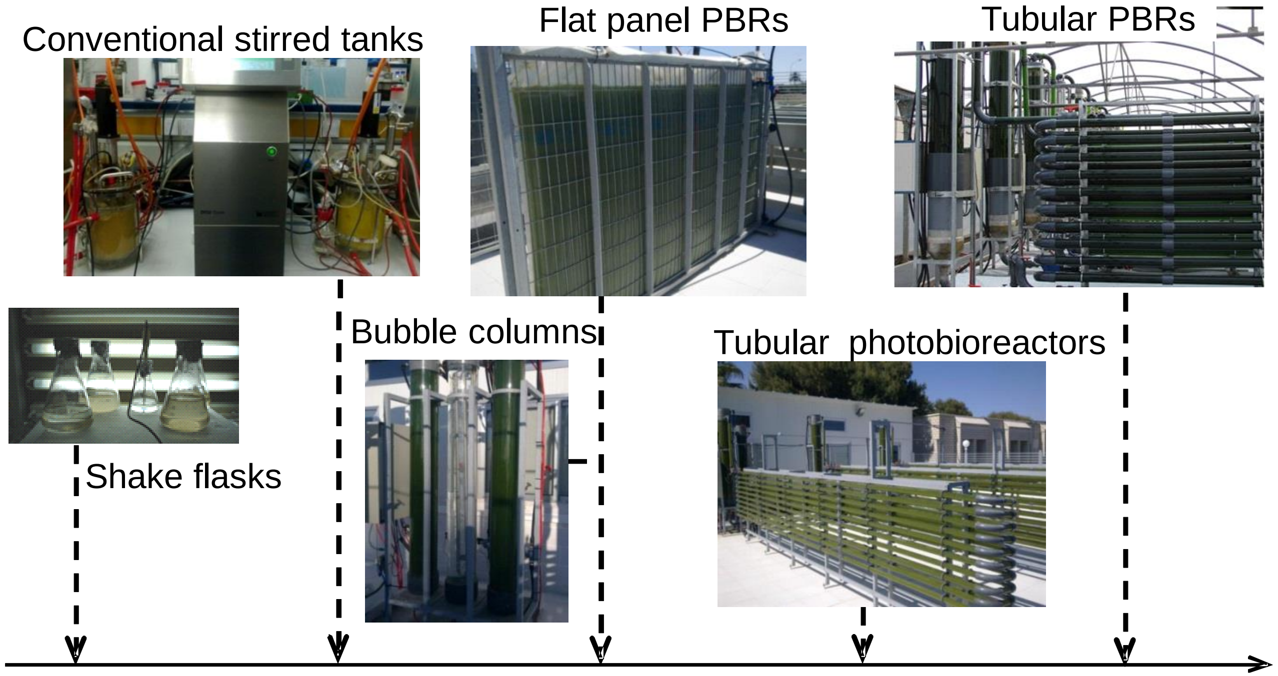
2. OBJECTIVES



Volume 0.1–1 1–20 10–10^2 50–500 >10^3

Biomass 1-10 g L^{-1} 1-10 Kg m^{-3}

0.01-0.3 g L^{-1} 1-300 g m^{-3}

Photobioreactors typically used for culturing **non-dinoflagellate microalgae** at different scales of operation

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How can bioactives from marine dinoflagellates become in drugs?

OPTIMIZATION UPSTREAM (US)
- Biomass productivity
- High titer

Cell Engineering
- Overproductive strain screening
- Metabolic engineering
- Expression technology
- …

Medium
- Essential nutrients
- Concentration
- Composition
- Shear protective additives
- Antifoam aspects
- …

Operation
- Batch
- Fed-Batch
- Semi/continuous cultivation
- Concentrated fed-batch
- …

Photobioreactor
- Type
- Fluiddynamics
- Shear stress
- Illumination
- Scale-up
- Biofouling
- …

Process monitoring
- Temperature
- pH
- Dissolved oxygen
- Nutrients
- Irradiance
- Gas transfer
- Product
- Side components
- …

OPTIMIZATION DOWNSTREAM (DS)
- Yield, purity
- Larger process capacities
- Faster process development

Separation Efficiency
- Harvesting
- Fractionation
- Adsorption
- Chromatography
- …

Process development
- Proof of concept
- Miniplant technology
- Continuous/batch processing
- …

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How can bioactives from marine dinoflagellates become in drugs?

1. Implementation of strategies for improving the **culture medium formulation**.
2. Analysis of **nutrient interactions** and evaluation of their **relative impact** on cell growth.
3. Study of **sensitivity** of dinoflagellate microalgae to turbulence developed in PBRs (energy dissipation rate, *EDR*).
4. Optimization of engineering factors influencing to **hydrodynamics** of bubblue column PBRs for shear-sensitivity microalgae.
5. **Pilot-plant scale-up** of the culture in pneumatically agitated PBRs (indoor /outdoor conditions).

3. Strategies

3.1.Improvement of culture media

López-Rosales, L., García-Camacho, F., Sánchez-Mirón, A., & Chisti, Y. (2015). An optimal culture medium for growing Karlodinium veneficum: progress towards a microalgal dinoflagellate-based bioprocess. Algal Research, 10, 177-182.

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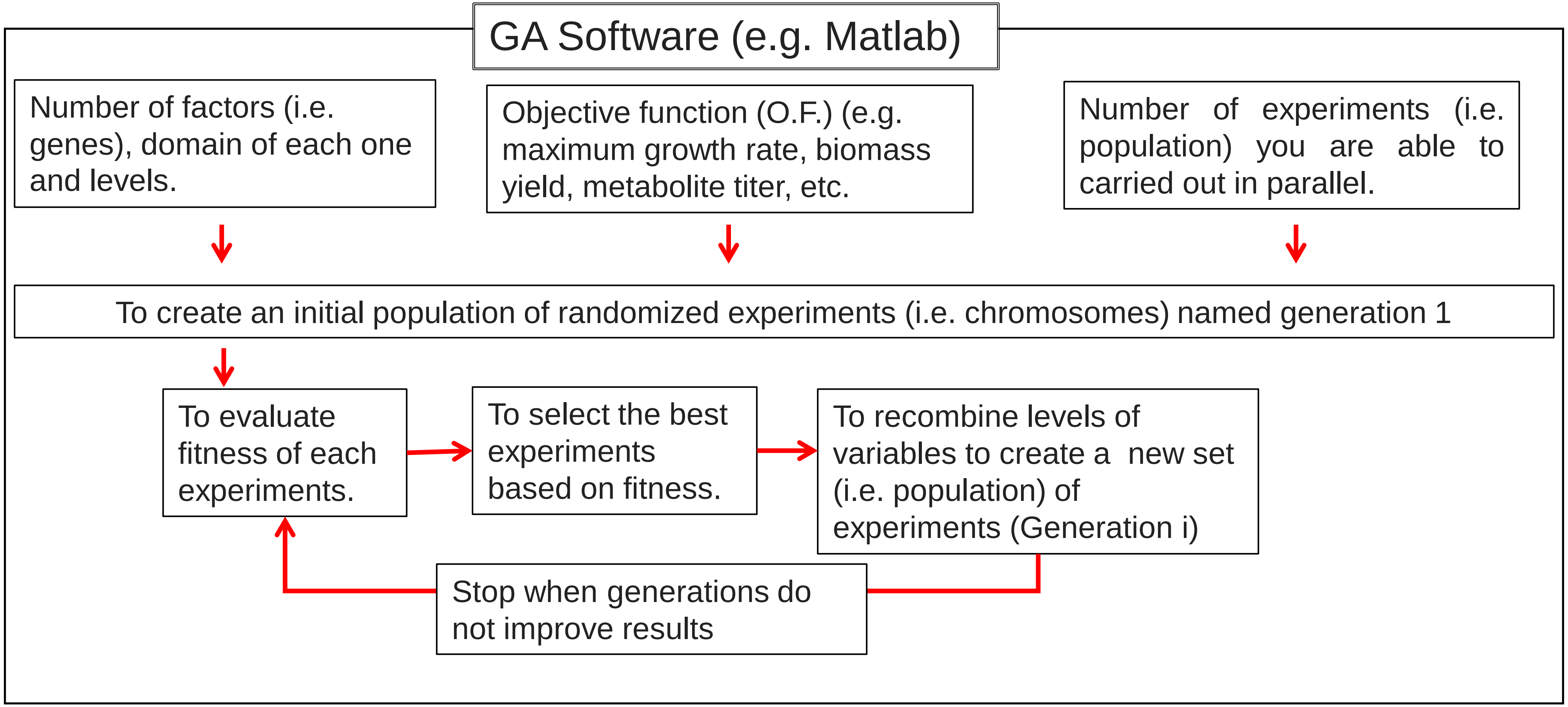
Improvement of culture media

OUTLINE

✓ **Genetic algorithms (GA).** A class of stochastic search strategies inspired by the process of natural selection. GA are commonly used to **optimization** and search problems by relying on bio-inspired operators such as mutation, crossover and selection



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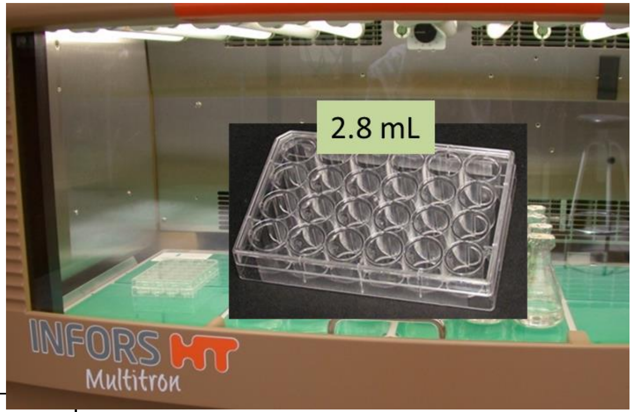
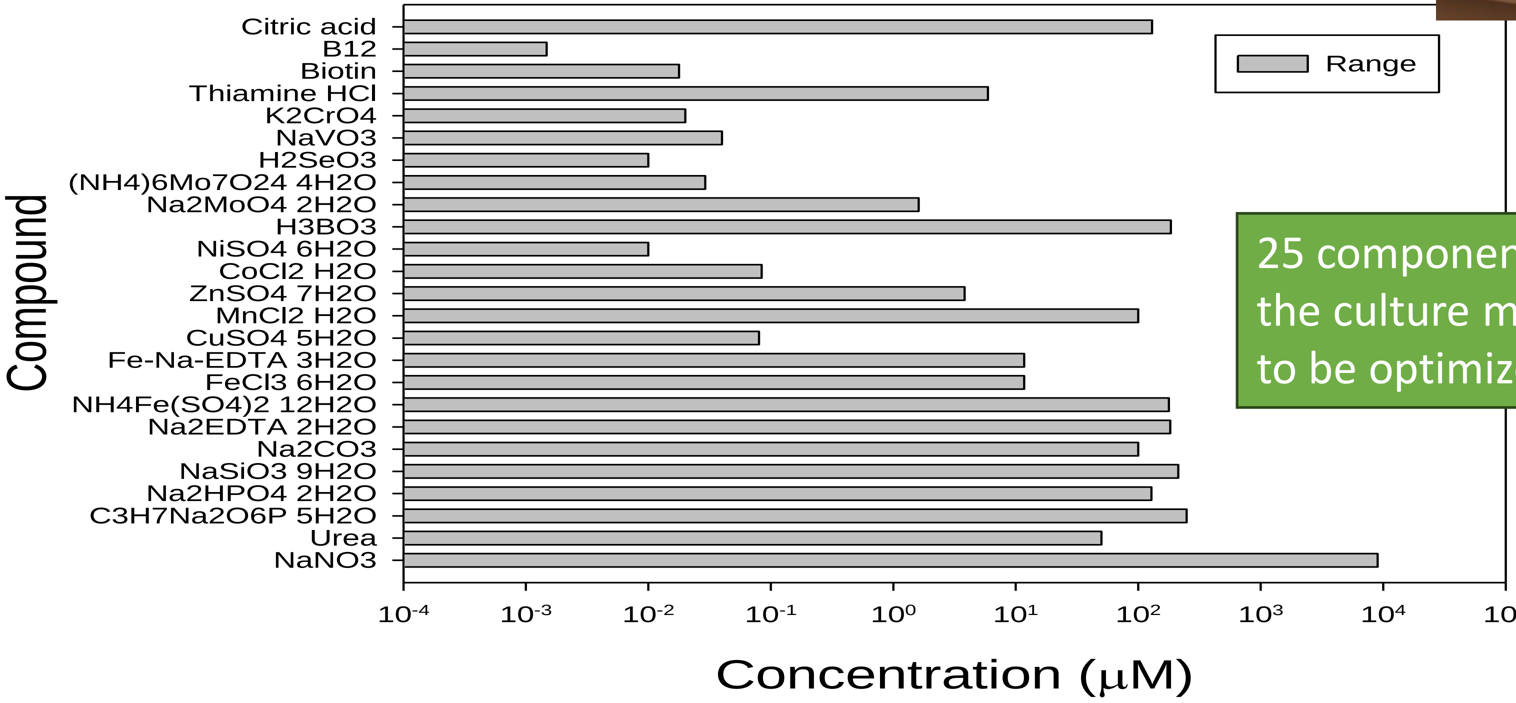
Improvement of culture media (*Karlodinium veneficum*)

Photobioreactors: Polystyrene multiwell plates (24-wells; Corning®) placed in incubator. Volume: 2.8 mL. Initial concentration: 3×10^4 cells·mL^{-1}. Irradiance: 200 µmol·m^{-2}·s^{-1} (12:12h light–dark regimen) from daylight fluorescent lights. Temperature: 18±1 ° The optimization experiments were in triplicate.





25 components of the culture medium to be optimize

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Improvement of culture media (*Karlodinium veneficum*)

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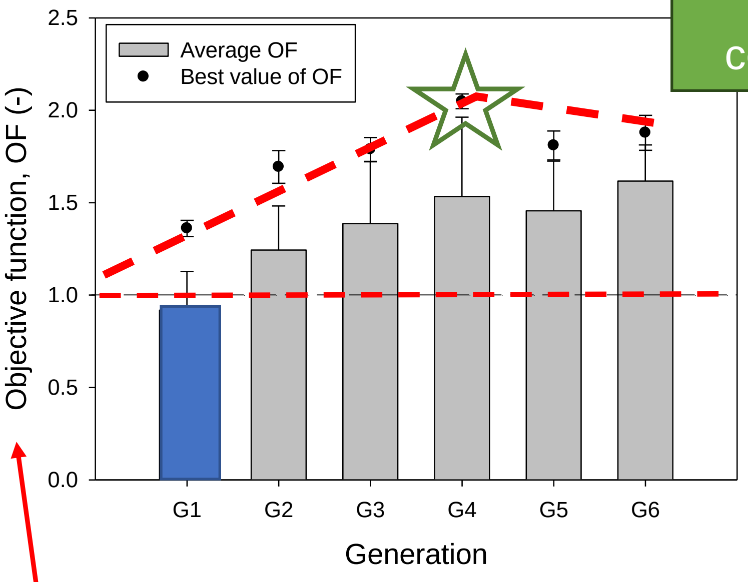
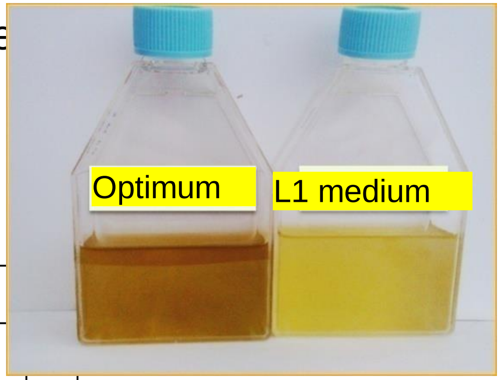
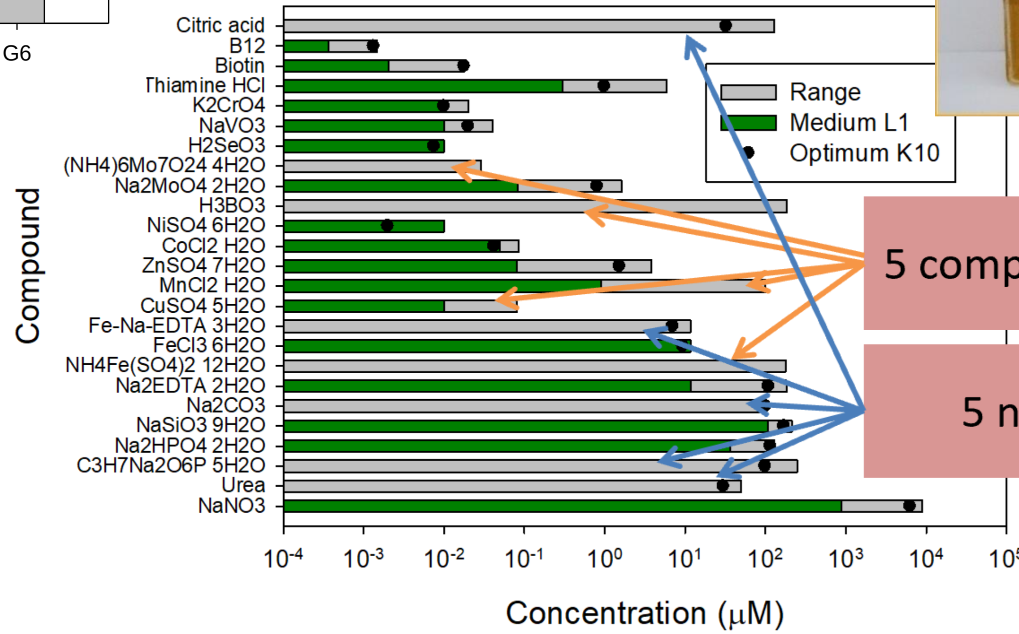


O.F= biomass productivity relative to the control culture grown in the L1 medium

Optimum composition

Maximum number of individuals (i.e. experiments) in a single generation= 70 cultures.
420 experiments, each with a different me



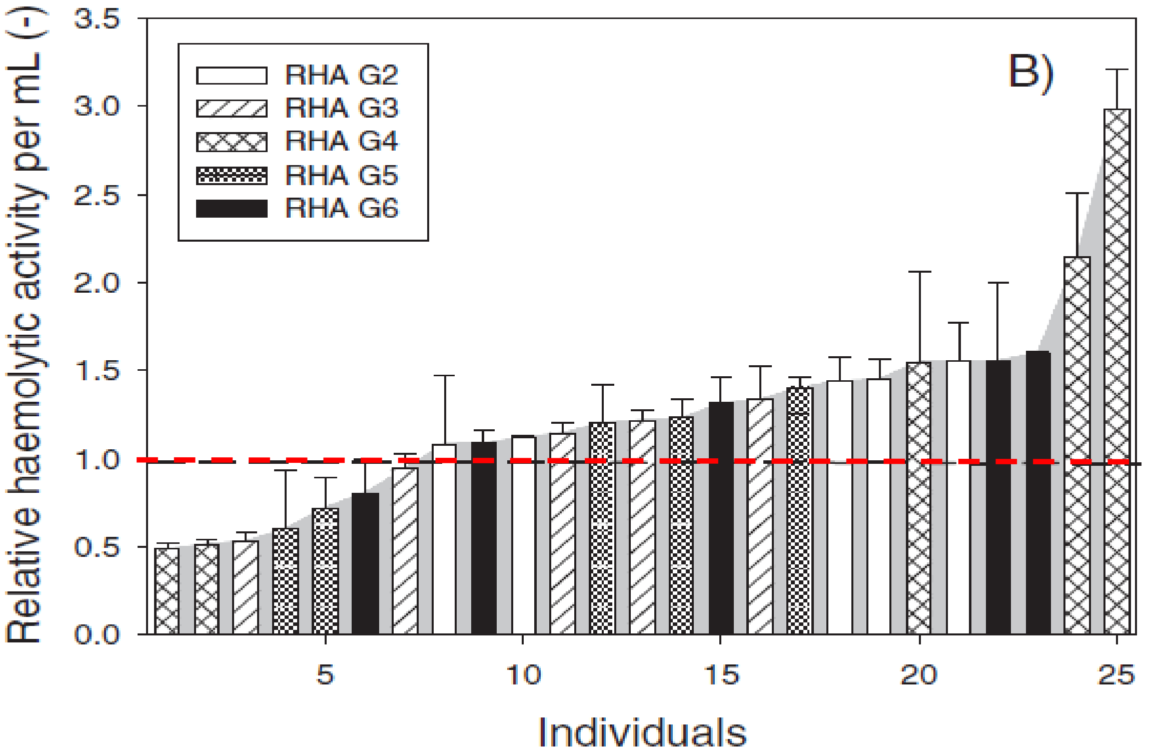


5 compounds were ruled out

5 new components

Improvement of culture media (*Karlodinium veneficum*)

OUTLINE



Most of them exceeded the control

Fig.. Relative hemolytic activity (RHA) per per milliliter of culture supernatan for the five best media formulations of each generation. The dashed line indicates the RHA for the L1 control culture.

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3. Strategies

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3.2. Modelling the growth of dinoflagellates with artificial neural networks

García-Camacho, F., **López-Rosales, L**., Sánchez-Mirón, A., Belarbi, E. H., Chisti, Y., & Molina-Grima, E. (2016). Artificial neural network modeling for predicting the growth of the microalga Karlodinium veneficum. Algal Research, 14, 58-64.

López-Rosales, L., Gallardo-Rodríguez, J. J., Sánchez-Mirón, A., Contreras-Gómez, A., García-Camacho, F., & Molina-Grima, E. (2013). Modelling of multi-nutrient interactions in growth of the dinoflagellate microalga *Protoceratium reticulatum* using artificial neural networks. Bioresource technology, 146, 682-688.

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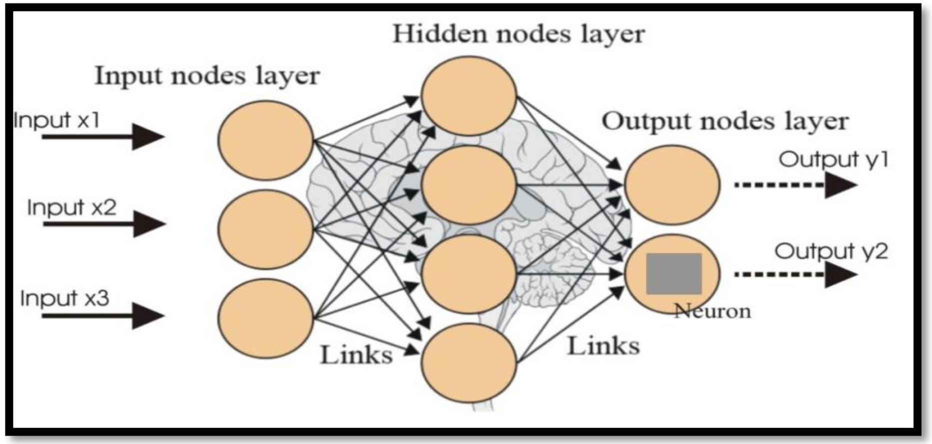
What does Artificial Neural Network (ANN) mean?

- It is a **computational model** based on the structure and functions of **biological neural networks**.
- ANNs solves problems that would prove impossible or difficult by human or statistical standards
- ANN has **self-learning capabilities** that enable it produce better results as more data becomes available.
- The **structure** of the ANN is function of Information that flows through it (i.e. **typology of the inputs and outpus**)
- ANNs are considered **nonlinear statistical data modeling tools** where the **complex** relationships between inputs and outputs are modeled or patterns are found.



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OUTLINE

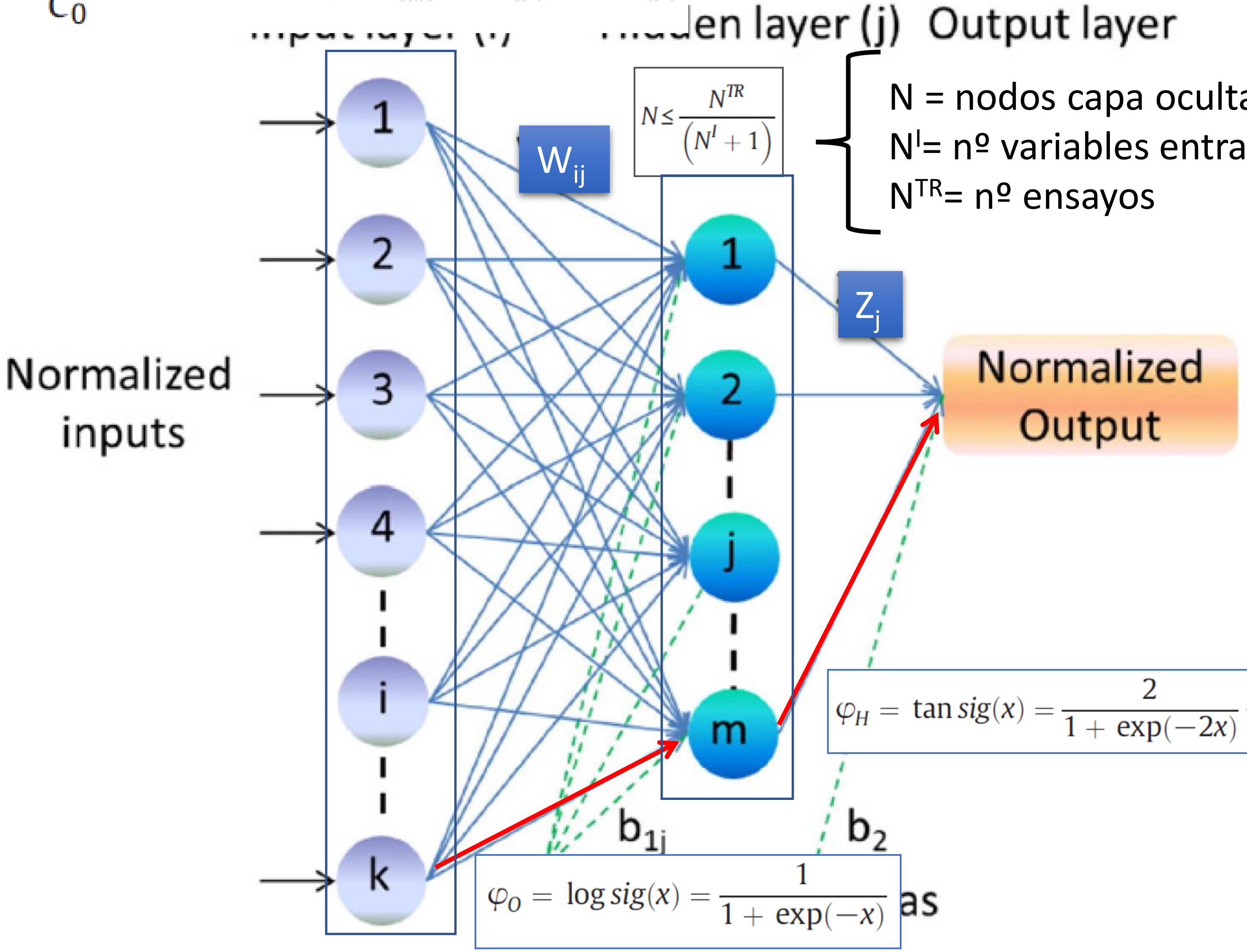
$$y_N = \varphi_O\left(\sum_{j=1}^{m} Z_j \cdot \varphi_H\left(\sum_{i=1}^{k} W_{ij} \cdot I_i + b_{1j}\right) + b_2\right)$$

es de
ación

$$y_N = 2\frac{y - y_{min}}{y_{max} - y_{min}} - 1$$

I_N

$$\frac{C_t}{C_0} = 0.5\,(y_N + 1)(y_{max} - y_{min}) + y_{min}$$



$$N \le \frac{N^{TR}}{(N^I + 1)}$$

N = nodos capa oculta

N^I= nº variables entrada

N^{TR}= nº ensayos

$$\varphi_H = \tan sig(x) = \frac{2}{1 + \exp(-2x)} - 1$$

$$\varphi_O = \log sig(x) = \frac{1}{1 + \exp(-x)}$$

as

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ANNmodeling for predicting the growth of the microalga *Karlodinium veneficum*

OUTLINE

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ANN input variable. a matrix (k x m) whose columns were vectors with the concentrations (µM) of 25 components selected, initial cell concentration (C0) and culture time (t).

Target variable: vector with C_t/C_0.

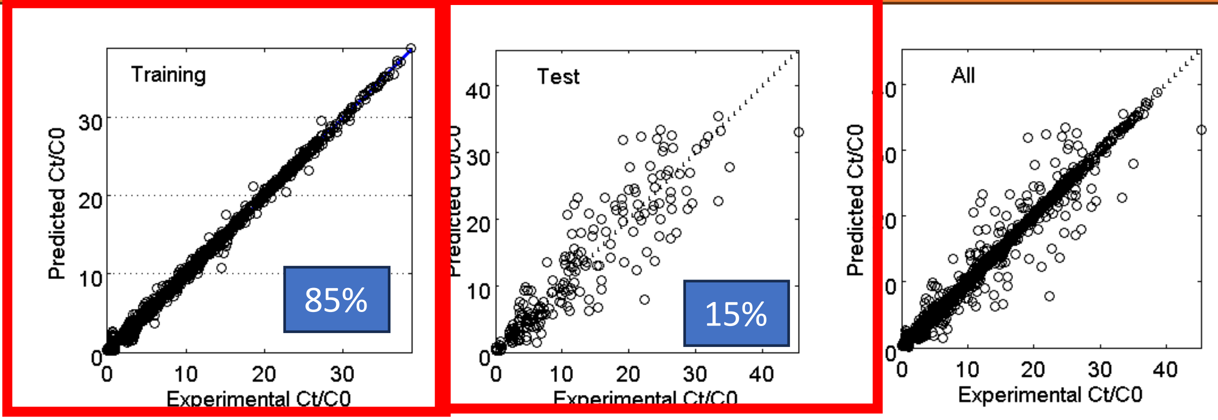
Implementation of the ANN model formulation in Matlab

Generation	1	1	1	1	1	5	5	5	5	5	5	5	5	5
t, days	0	0	6	6	6	3	3	3	3	6	6	6	6	6
[Co], cells/mL	33000	31333.3333	32666.6667	33000	33666.6667	27666.6667	30333.3333	28666.6667	31000	30333.3333	31000	28666.6667	29000	32000
$NaNO_3$	0	0	2700	5400	3600	900	8100	2700	882	1800	8100	3600	4500	6300
Urea	20	20	30	30	10	20	30	20	0	20	10	30	50	40
Glicerophosphate	99.2	148.8	74.4	24.8	49.6	74.4	173.6	74.4	0	173.6	173.6	173.6	99.2	74.4
PO_4^{-3}	64	115.2	25.6	12.8	12.8	89.6	115.2	128	36.2	76.8	128	12.8	102.4	115.2
SiO_4^{-2}	42.4	42.4	169.6	42.4	169.6	42.4	84.8	42.4	106	127.2	127.2	169.6	42.4	84.8
CO_3^{-2}	60	20	20	80	20	100	20	100	0	60	60	20	60	100
$Na_2EDTA·2H_2O$	36.46	109.38	182.3	0	145.84	109.38	0	0	11.7	145.84	145.84	36.46	36.46	145.84
Fe(NH4)2(SO4)2·6H20	0	89	178	89	44.5	0	133.5	44.5	0	44.5	44.5	0	0	0
$FeCl_3·6H_2O$	4.68	11.7	7.02	2.34	9.36	9.36	11.7	0	11.7	0	0	7.02	4.68	9.36
$Fe-Na-EDTA·3H_2O$	7.02	2.34	2.34	7.02	2.34	7.02	4.68	9.36	0	7.02	7.02	7.02	7.02	4.68
$CuSO_4·5H_2O$	0.06	0.04	0.04	0.06	0.06	0	0.04	0.04	0.01	0.04	0.04	0.04	0.06	0.04
$MnCl_2·H_2O$	20	60	80	70	70	0	90	30	0.91	30	30	0	20	50
$ZnSO_4·7H_2O$	2.674	3.056	1.91	3.82	2.292	1.528	0.382	3.438	0.08	2.292	2.292	1.528	2.674	2.292
$CoCl_2·6H_2O$	0.056	0.056	0.042	0.07	0.056	0.042	0.056	0.028	0.05	0.014	0.042	0.014	0.056	0.014
$NiSO_4·6H_2O$	0.008	0.002	0.004	0.01	0.006	0.008	0.002	0.01	0.01	0	0.002	0.006	0.008	0.004
H_3BO_3	74	74	111	148	37	111	74	185	0	148	0	111	74	111
$Na_2MoO_4·2H_2O$	1.072	0.804	0	1.34	0	1.34	0.268	1.072	0.0822	1.072	0.804	0.536	1.072	0.536
$(NH_4)_6Mo_7O_{24}·4H_2O$	0.00728	0.01456	0.02184	0.01456	0	0.02912	0.02912	0.02184	0	0.02184	0	0.00728	0.00728	0.02184
H_2SeO_3	0.005	0.01	0.005	0.01	0.01	0.005	0	0	0.01	0.005	0.01	0.01	0.005	0.0025
Na_3VO_4	0.02	0.03	0.01	0.02	0.03	0.03	0	0.02	0.01	0.03	0.02	0.03	0.02	0.03
K_2CrO_4	0	0.01	0.02	0.015	0.02	0.02	0	0.01	0.01	0	0	0.01	0	0
Thiamine·HCl	0	2.964	0	4.94	4.94	2.964	0.988	4.94	0.296	3.952	0.988	0	0	2.964
Biotin	0.00712	0.01246	0.01424	0.01424	0.00356	0.01068	0.00356	0.01424	0.002045	0.01424	0.00356	0.01424	0.00712	0.00356
Vitamin B_{12}	0.000296	0.000296	0	0.000296	0.000888	0.000296	0.001332	0.000888	0.000369	0.001184	0.000592	0.00074	0.000296	0.000888
Citric Acid·H_2O	32.5	130	0	65	65	97.5	97.5	97.5	0	32.5	97.5	65	32.5	97.5

m= 1398

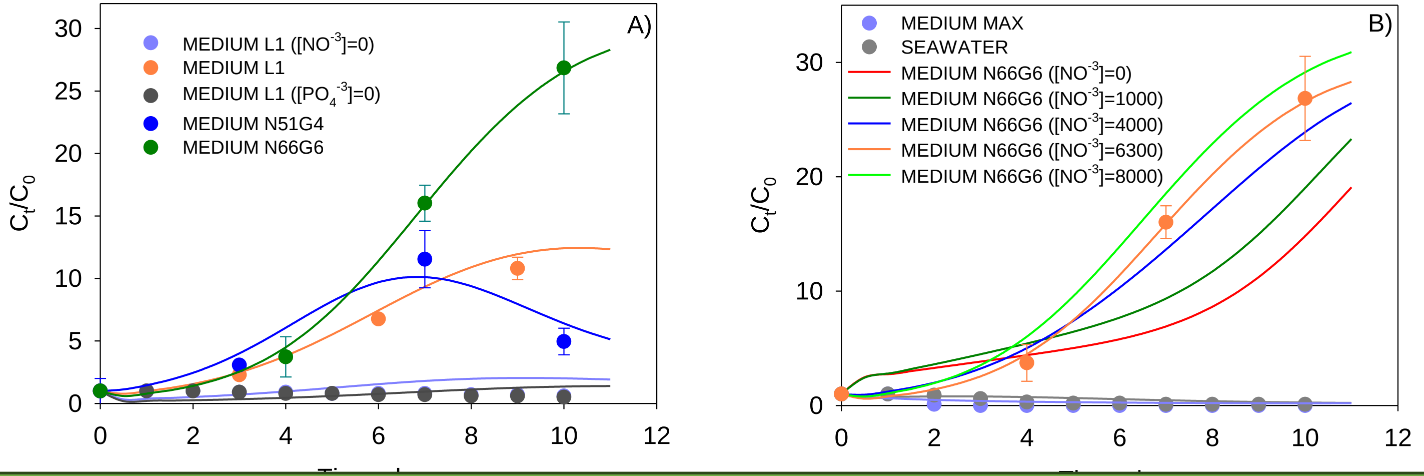
k=27

ANNmodeling for predicting the growth of the microalga *Karlodinium veneficum*



R > 0.984

A parity plot comparison of the predicted and the measured (experimental) dimensionless cell concentrations for the training runs, the test runs and all the runs combined.



Examples of growth kinetics simulated by FBN model for different formulations of culture media**Solids circles represent experimental data. Predictions of the FBN model are shown as solid lines.**

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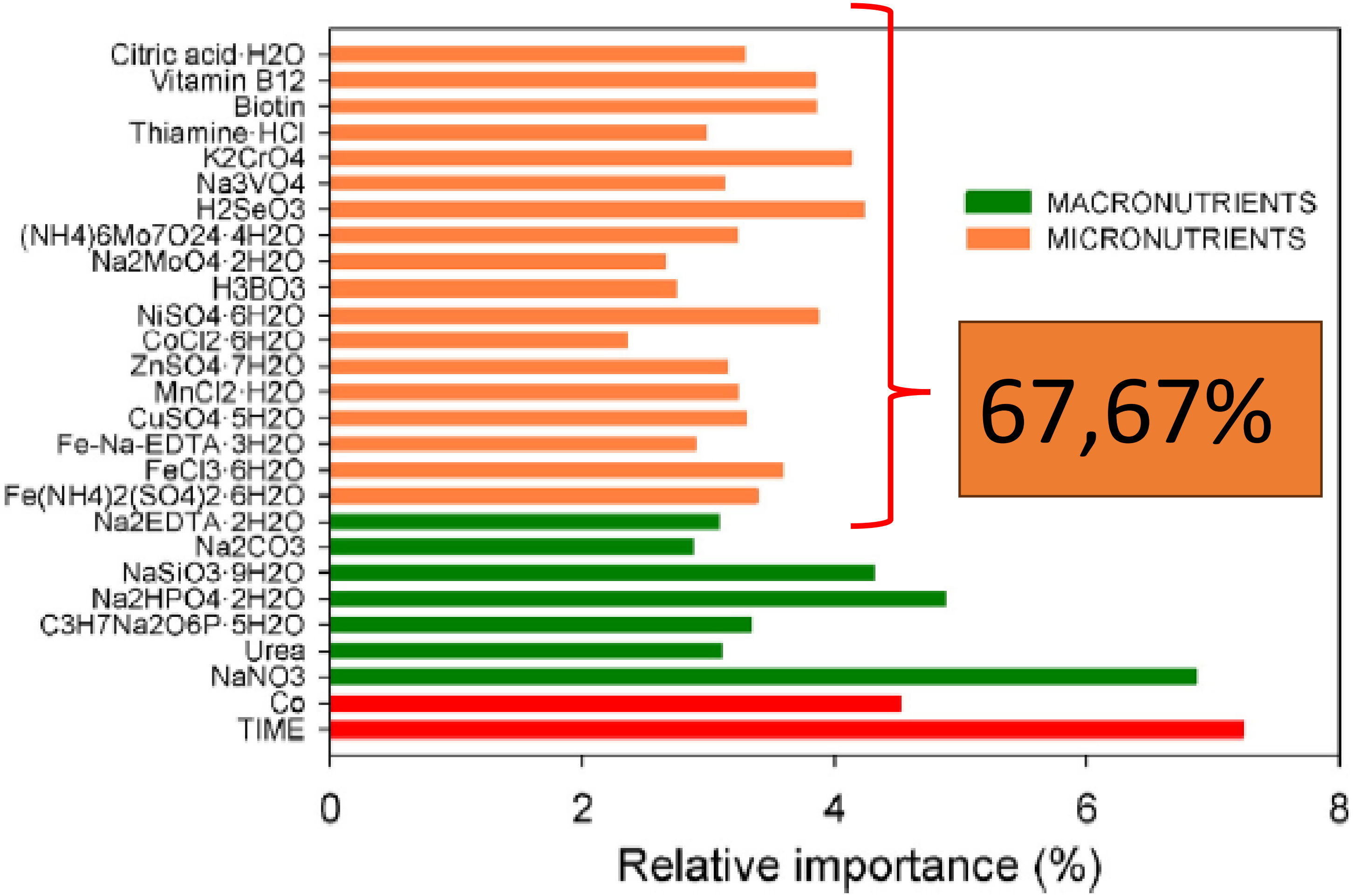
ANNmodeling for predicting the growth of the microalga *Karlodinium veneficum*

OUTLINE



67,67%

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3. Strategies

3.3.Optimization of culture conditions

López-Rosales, L., García-Camacho, F., Sánchez-Mirón, A., Contreras-Gómez, A., & Molina-Grima, E. (2015). An optimisation approach for culturing shear-sensitive dinoflagellate microalgae in bench-scale bubble column photobioreactors. Bioresource technology, 197, 375-382.

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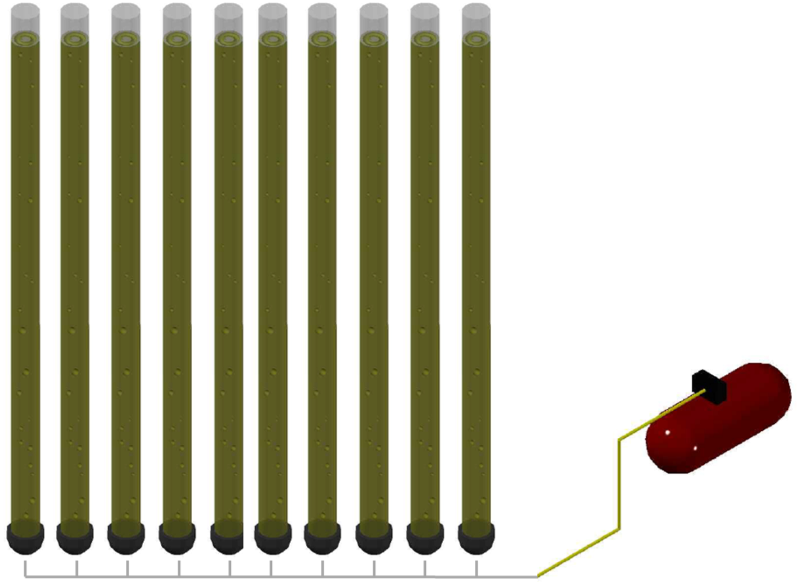
An optimisation approach for culturing *K. veneficum* in bench-scale bubble column PBRs

OUTLINE

✓ **Bank of bench-scale bubble column photobioreactors** (4.4 cm internal diameter) to find an optimal combination of values for the culture parameters gas flow rate, culture height, and nozzle sparger diameter

Factor	Units	Range of variation	Step-width
Air flow rate (*Q*)	$L\ min^{-1}$	0.1–0.5	0.13 (4)
Culture height (*H*)	m	0.50–1.75	25 (6)
Nozzle diameter (d_0)	mm	1–2.5	0.5 (4)

The numbers in parentheses represent the number of possible levels for each factor. The product of these values gives the total number of possible experiments ($4 \times 6 \times 4 = 96$).



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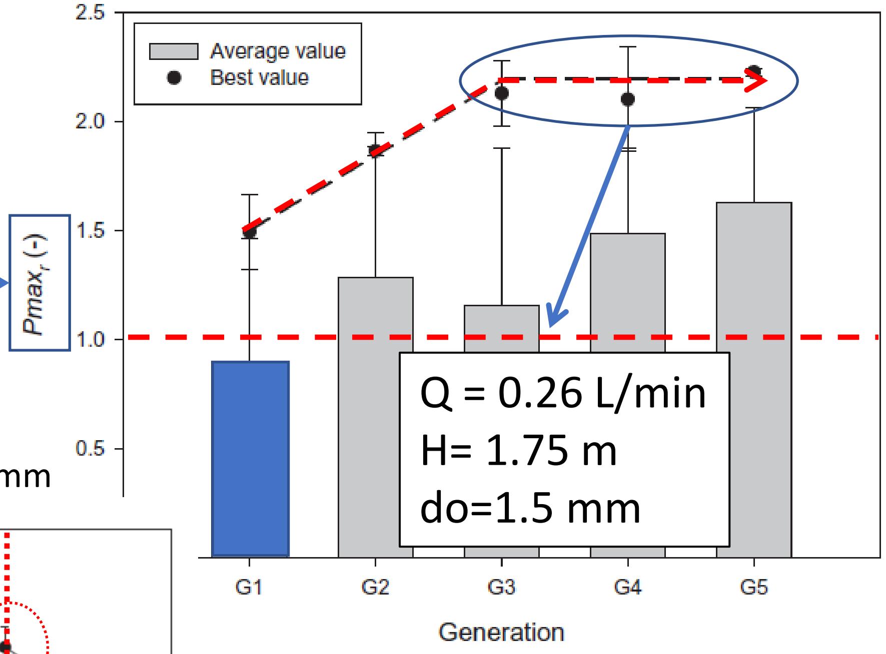
An optimisation approach for culturing *K. veneficum* in bench-scale bubble column PBRs

OUTLINE

Optimization technique: Genetic algorithm → Number of individuals (i.e. experiments) in a single generation= 5 batch cultures.

25 experiments, each with a different combination of Q, H and d_o.

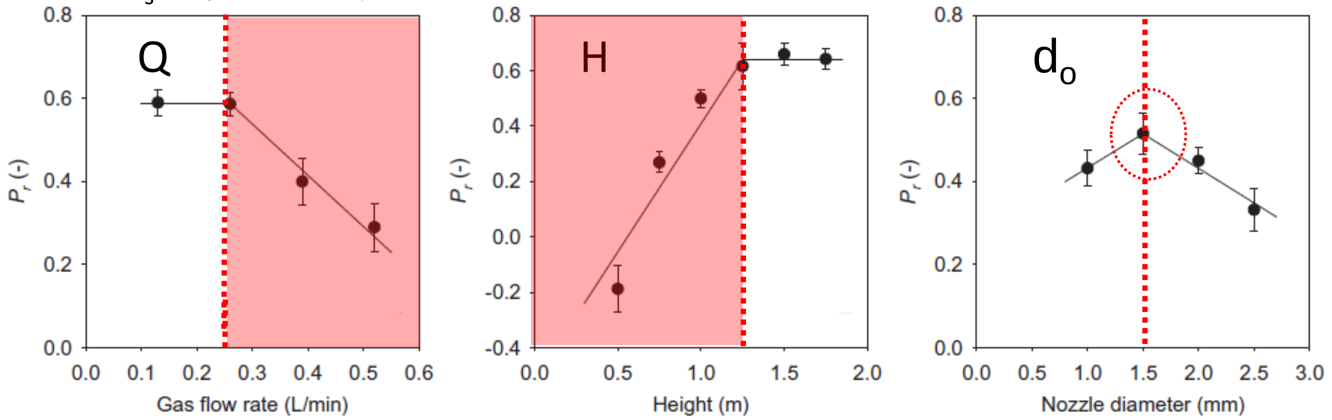
O.F.= maximum biomass productivity ($Pmax_r$) relative to the static control culture in T-Flask



Q < 0.26 L/min
or 0,137 vvmin
$v_s < 2{,}85 \cdot 10^{-3}$ m/s

H> 1.25 m

do=1.5 mm



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An optimisation approach for culturing *K. veneficum* in bench-scale bubble column PBRs

OUTLINE

1. Overview
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3.1 Improvement of culture media

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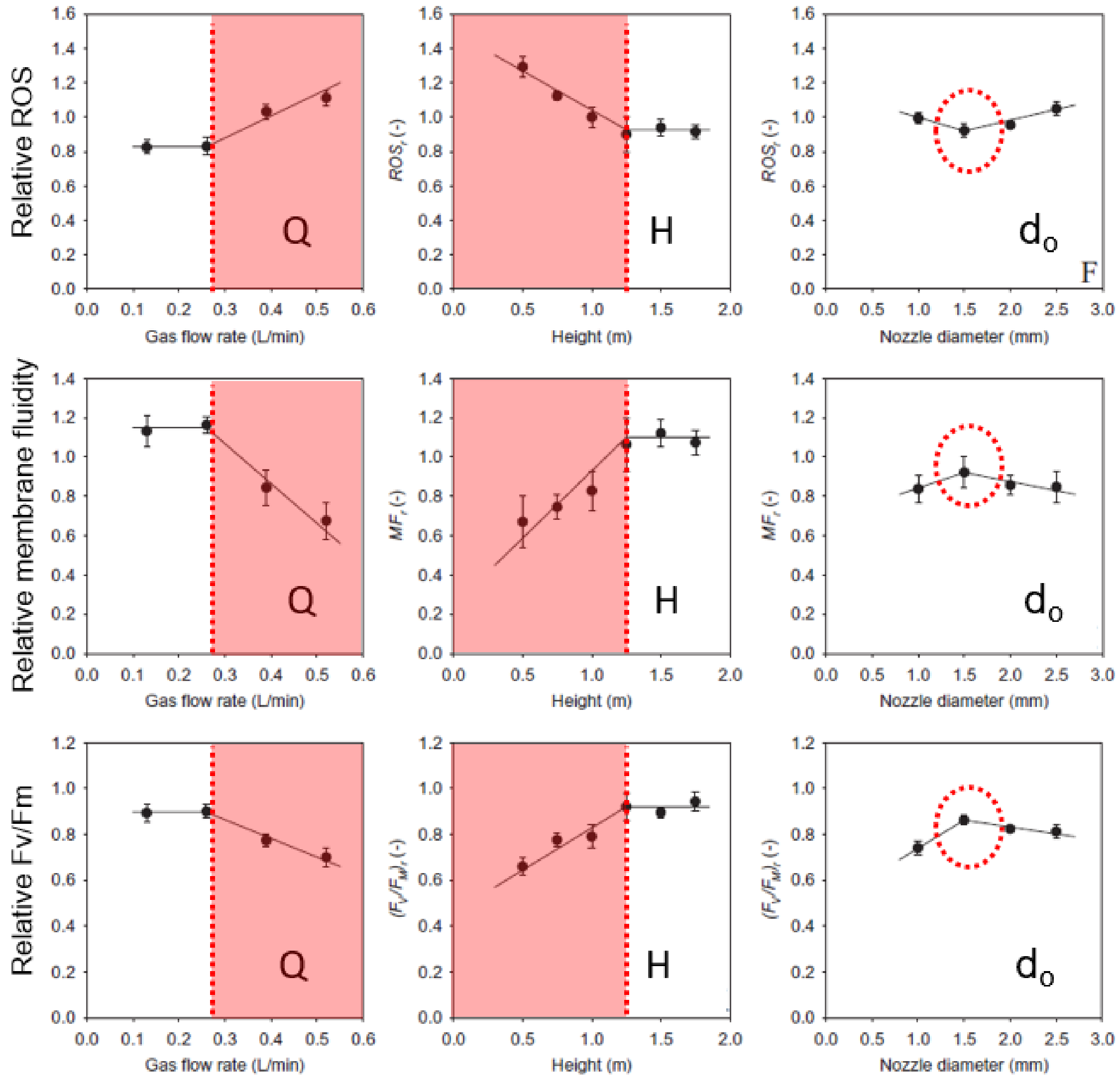
4. Conclusions



ROS ↑ MF ↓ Fv/Fm ↓

Damage by hydrodynamic stress

Work window

Q(Lpm)	H(m)	do(mm)
<0.26	>1.25	1.5

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3. Strategies

OUTLINE

3.4. Recycling recourses

L. López-Rosales, P. López-García , M.A. Benyachou , A. Molina-Miras , J.J. Gallardo-Rodríguez , M.C. Cerón-García , A. Sánchez Mirón , F. García-Camacho(2022). Treatment of secondary urban wastewater with a low ammonium-tolerant marine microalga using zeolite-based adsorption. Bioresource Technology 359

A Molina-Miras**, L López-Rosales,** MC Cerón-García, A Sánchez-Mirón**, A Olivera-Gálvez**, F García-Camacho, Molina-Grima (2020) .Acclimation of the microalga Amphidinium carterae to different nitrogen sources: potential application in the treatment of marine aquaculture effluents. Journal of applied phycology

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Recycling recourses: Use of urban wastewater

OUTLINE

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Journal of Applied Phycology
https://doi.org/10.1007/s10811-020-02049-9

Check for updates

Acclimation of the microalga *Amphidinium carterae* to different nitrogen sources: potential application in the treatment of marine aquaculture effluents

A. Molina-Miras[1] · L. López-Rosales[1] · M. C. Cerón-García[1] · A. Sánchez-Mirón[1] · A. Olivera-Gálvez[2] · F. García-Camacho[1] · E. Molina-Grima[1]

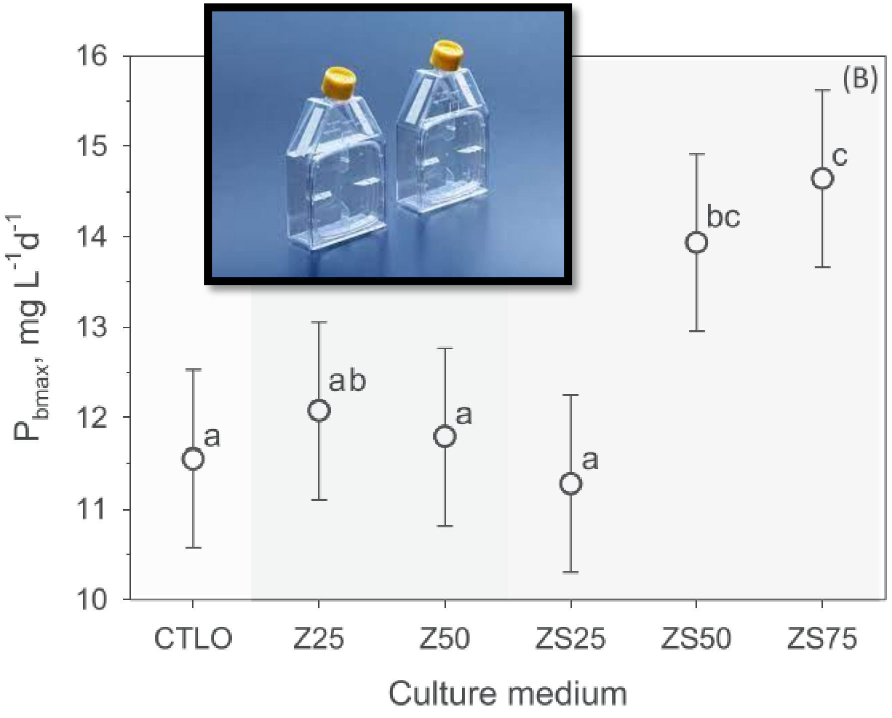
The initial treatment of wastewater is necessary to lower the concentration of ammoniacal nitrogen.

$NH4^{+}$-N_{UWW}=54mg/L

$NH4^{+}$-$N_{tox\approx}$≈ 6mg/L

Recycling recourses: Use of urban wastewater

Characteristics	Culture media CTLO	CTL1	CTL2	CTL3	CTL4	ZS25	Z25	ZS50	Z50	ZS75	Z75	ZS100	Z100	UWWS	UWW
UWW (%)	–	–	–	–	–	–	–	–	–	–	–	–	–	100	100
Zeolite-treated UWW (%)	–	–	100	50	–	25	25	50	50	75	75	100	100	–	–
Seawater (%)	100	100	–	50	–	75	75	50	50	25	25	–	–	–	–
Zeolite-treated SW (%)	–	–	–	–	100	–	–	–	–	–	–	–	–	–	–
Salinity Adjustment	No	No	Yes	Yes	No	Yes	No	Yes	No	Yes	No	Yes	No	Yes	No
Salinity (‰)	38.0	38.0	38.0	38.0	38.0	38.0	28.9	38.0	19.7	38.0	10.6	38.0	1.4	38.0	1.4
$[NH_4^+\text{-}N]_o$, mg L^{-1}	0.0	0.0	0.0	0.0	0	2.3	2.3	4.6	4.6	7.0	7.0	9.3	9.3	54.4	54.4
$[NO_3^-\text{-}N]_o$, mg L^{-1}	12.3	0.0	0.0	0.0	12.3	12.3	12.3	12.3	12.3	12.3	12.3	12.3	12.3	2.1	2.1
N_{T0}, mg L^{-1}	12.3	0.0	0.0	0.0	12.3	14.6	14.6	16.9	16.9	19.3	19.3	21.6	21.6	56.5	56.5
$[PO_4^{-3}\text{-}P]_o$, mg L^{-1}	5.4	0.0	0.0	0.0	5.4	5.4	5.4	5.4	5.4	5.4	5.4	5.4	5.4	0.8	0.8
$[TOC]_o$, mg L^{-1}	11.2	10.8	23.2	17	13.9	14.6	14.6	18	18	21.5	21.5	24.9	24.9	31.6	31.6



- No statistically significant differences between ZS50 and ZS75
- Without growth in the media that does not adjust the saline content
- The culture medium formulated with 75% urban wastewater treated with zeolites (7 mg/L NH_4^+-N) adjusting the salinity obtained the best results in productivity of maximum biomass generated

Recycling recourses: Use of urban wastewater

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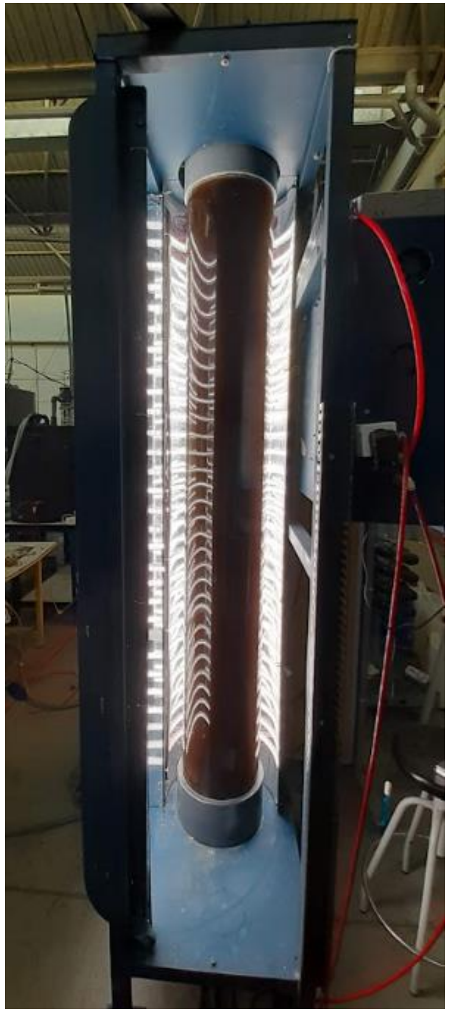

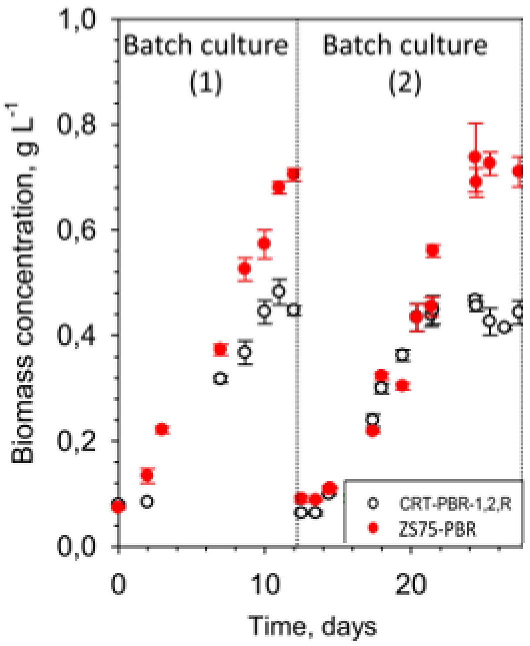

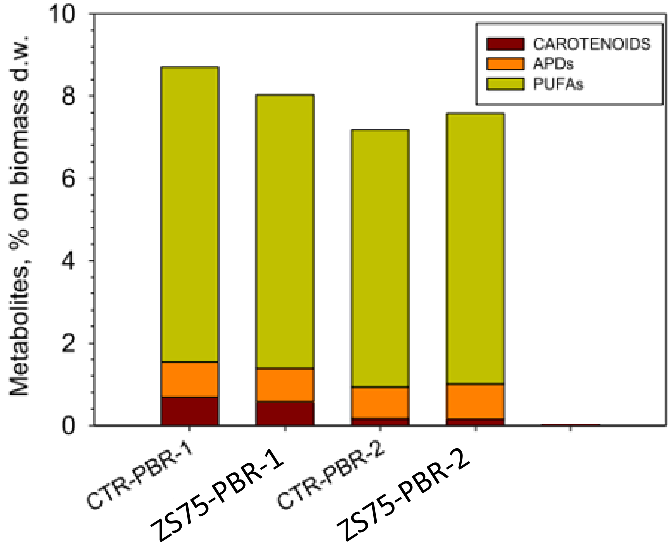

- Significant increase in biomass compared to the control culture
- No differences between the control culture and ZS75 in terms of dry weight percentage of biocompounds, but in terms of productivity

Recycling recourses: Use of urban wastewater

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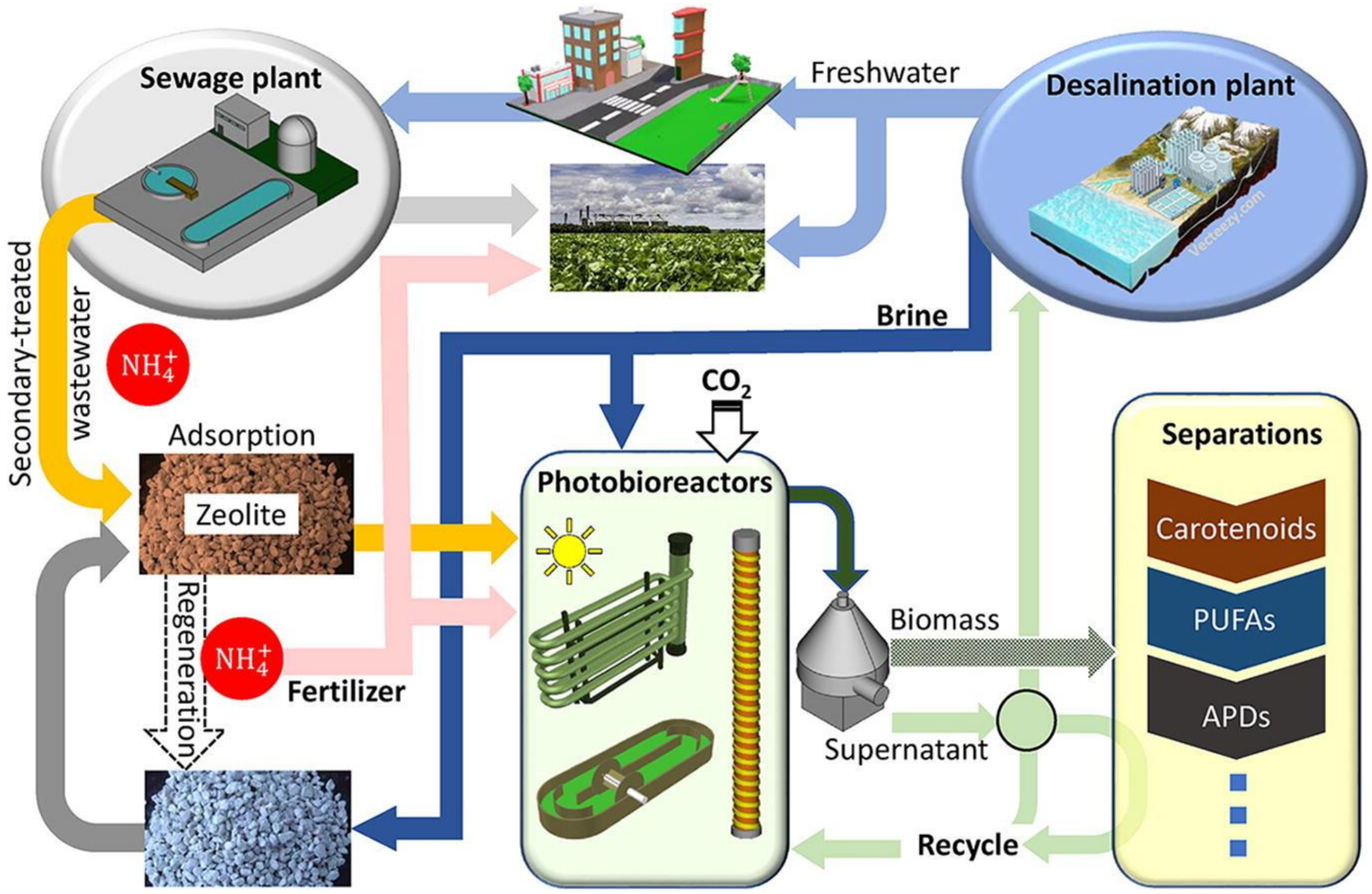

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3. Strategies

3.5. Scale-up of the bioprocess

López-Rosales, L., García-Camacho, F., Sánchez-Mirón, A., Contreras-Gómez, A., & Molina-Grima, E. (2015). An optimisation approach for culturing shear-sensitive dinoflagellate microalgae in bench-scale bubble column photobioreactors. Bioresource technology, 197, 375-382.

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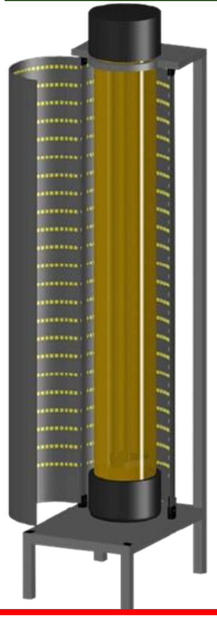
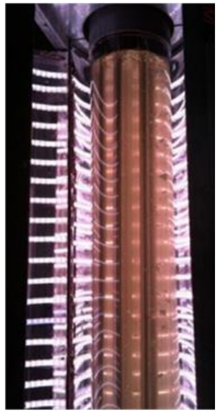
Pilot-scale photobioreactors illuminated with LEDs

OUTLINE

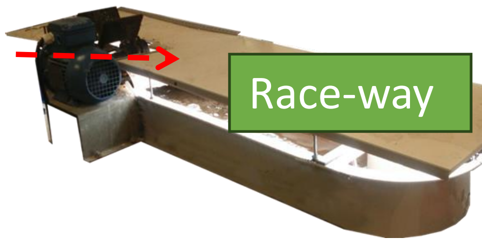
Bubble column





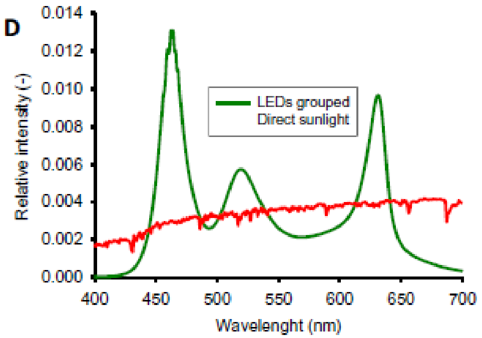
L. López-Rosales et al., 2016

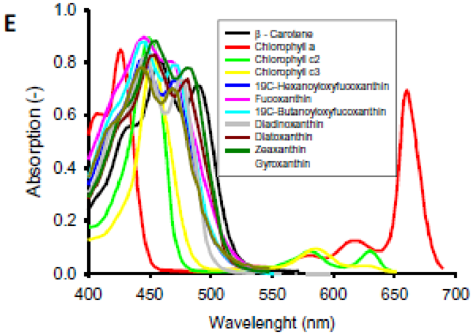
A. Molina-Miras et al., 2018b



Race-way

Each LED was a multi-chip LED with the ability to provide multiple colors.





Absorption spectra of all the key pigments matchs with the emission spectrum of the LEDs used. Therefore, LEDs had the potential to support algal photosynthesis

Criteria of scale-up of bubble column and flat panel PBRs

H > 1.25 m and $d_c/d_o \approx 20$ assured freedom from damaging levels of hydrodynamic stress so long as the superficial aeration velocity (v_s) remained below a species-dependent threshold value.

Pilot-scale outdoor flat panel photobioreactor

OUTLINE

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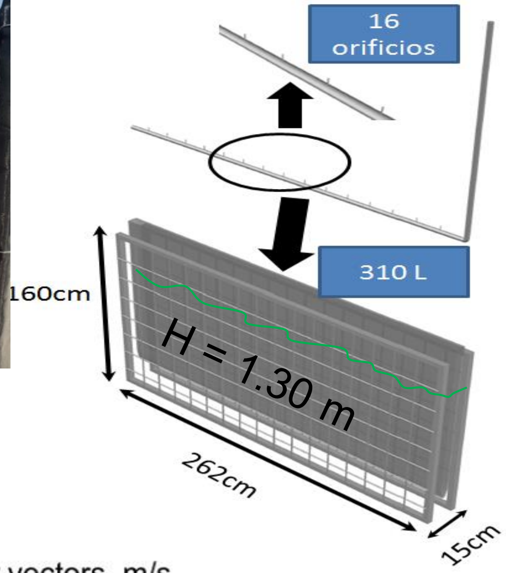
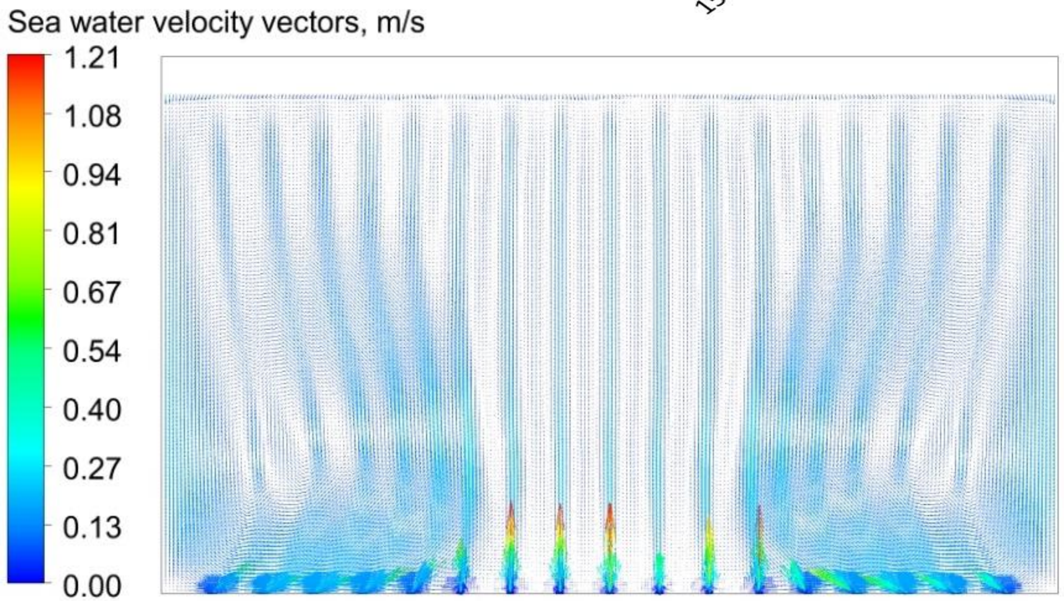
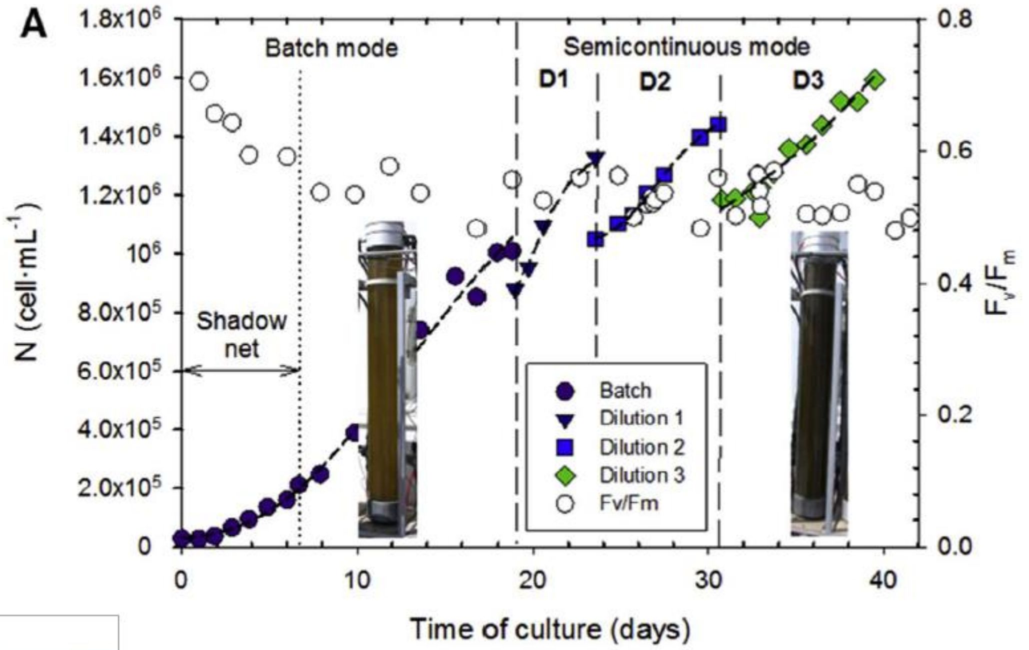
3.5 Scale-up of the bioprocess

4. Conclusions







L. López-Rosales et al., 2018

400L

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Some of the secondary metabolites isolated from our cultures so far

OUTLINE

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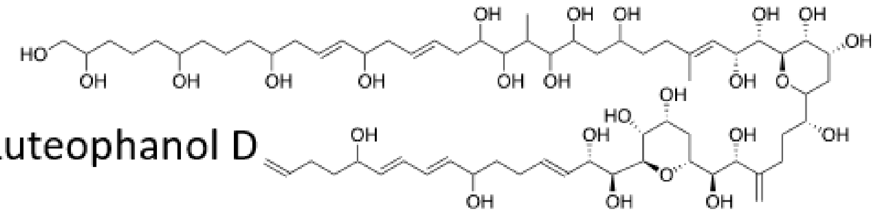
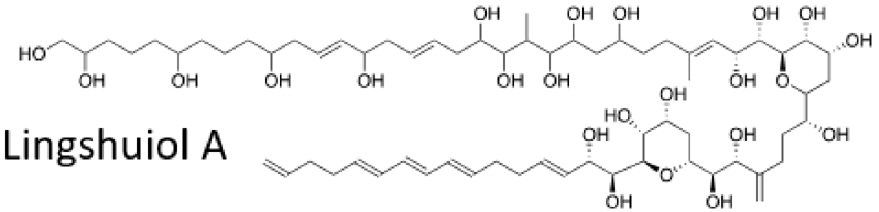
Amphidinium carterae

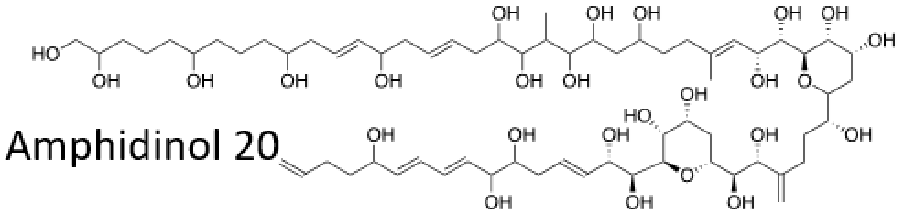
Luteophanol D



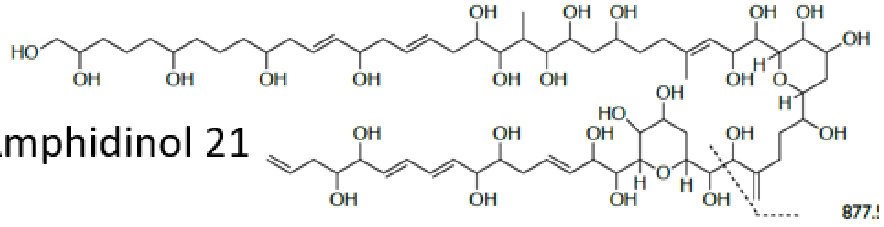
Lingshuiol A



Amphidinol 20

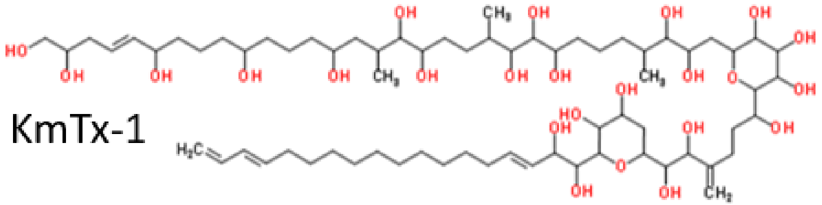


Amphidinol 21

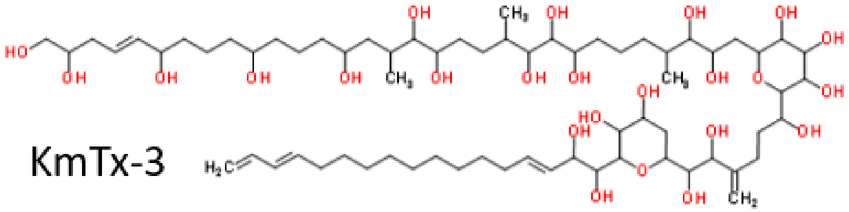


Karlodinum veneficum

KmTx-1



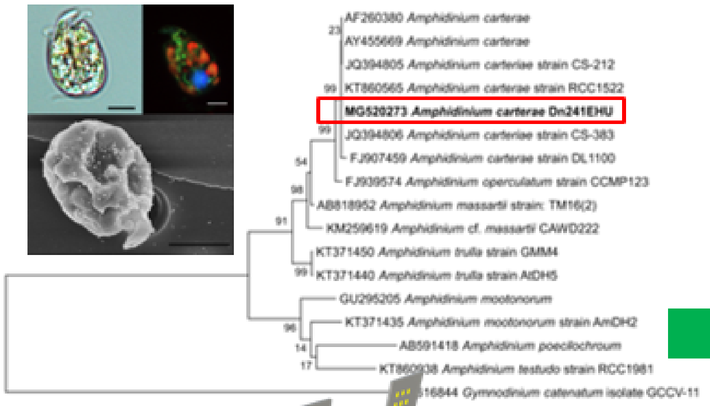
KmTx-3

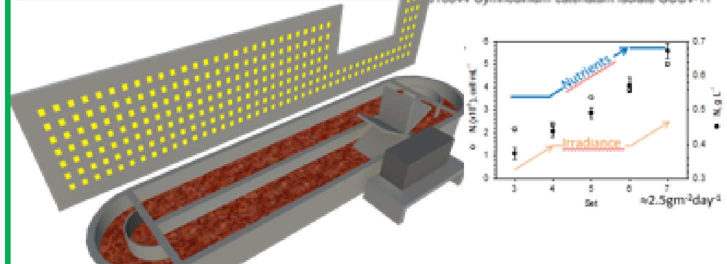


Compuond	m/z	Elemental composition
KmTx-1303	1303.73	$C_{62}H_{117}ClNaO_{22}S$
KmTx-1379	1379.75	$C_{64}H_{121}ClNaO_{25}S$
KmTx-1423	1423.68	$C_{62}H_{118}ClNa_2O_{26}S_2$
KmTx-1321	1321.74	$C_{62}H_{119}ClNaO_{23}S$
KmTx-1355	1355.75	$C_{62}H_{121}ClNaO_{25}S$

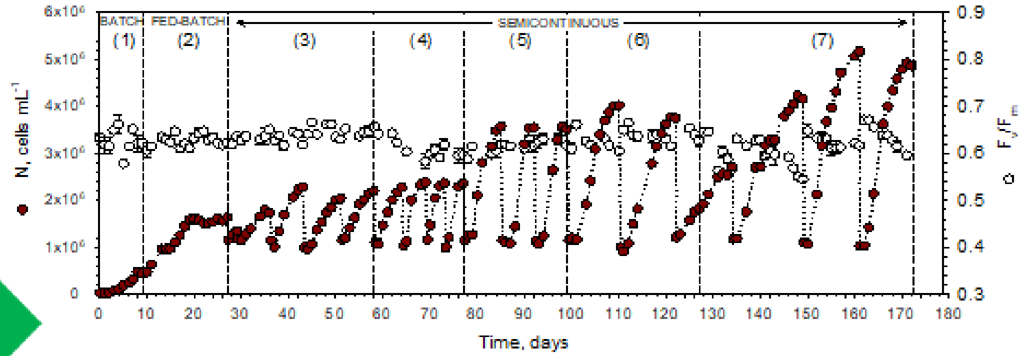
Example of *A. carterae* Dn241EHU , RW-PBR

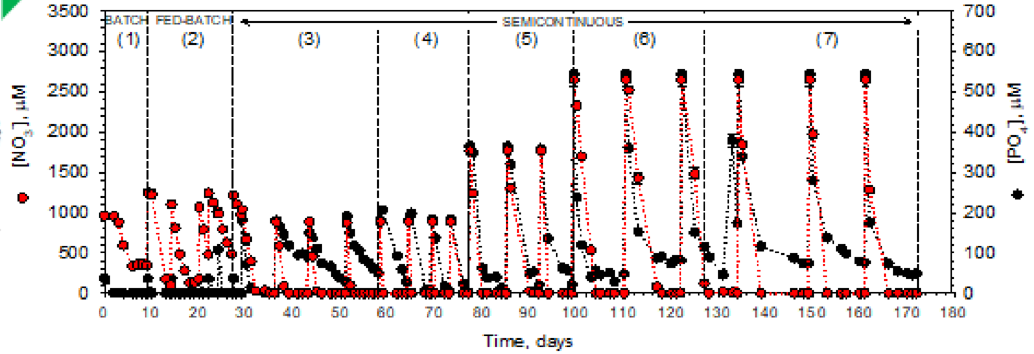
OUTLINE



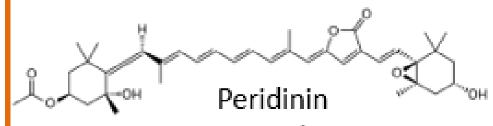


A. Molina-Miras et al., 2018b



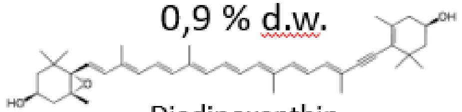


Carotenoids



Peridinin

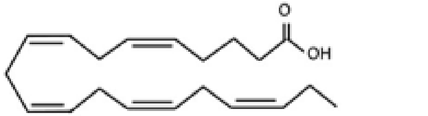
0,9 % d.w.



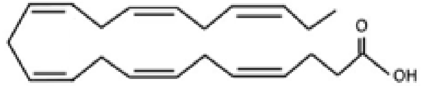
Diadinoxanthin

0,27 % d.w.

PUFAs



EPA 1,69% d.w.

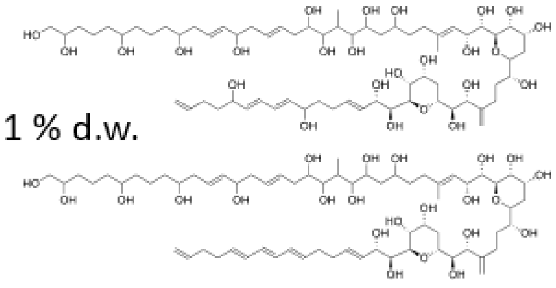


DHA 3,47% d.w.

Bioactive substances

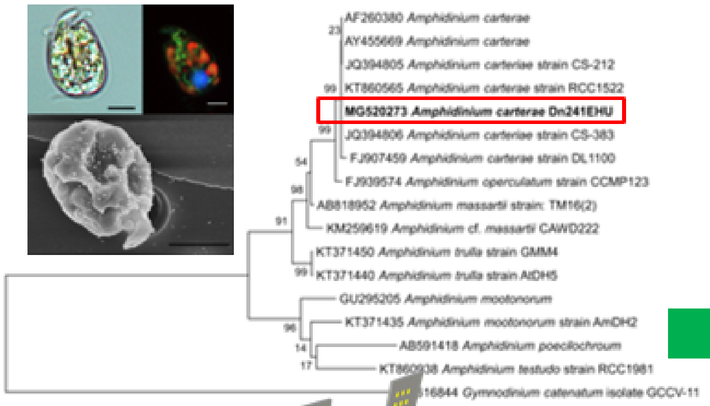
e.g. amphidinols

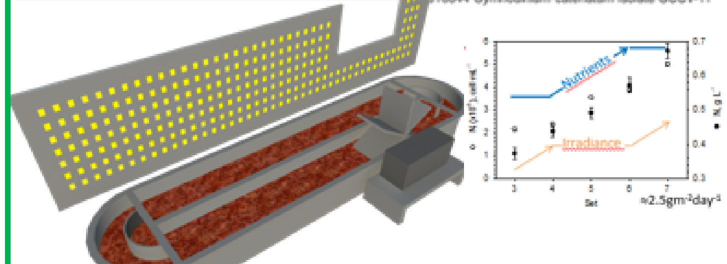
1 % d.w.



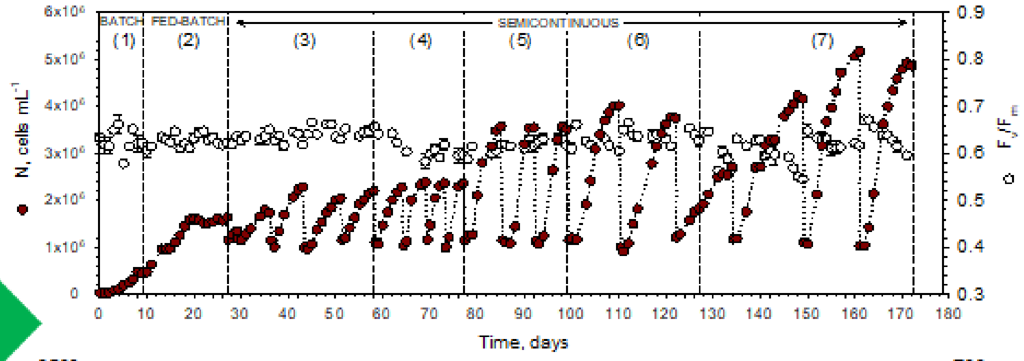
Example of *A. carterae* Dn241EHU , RW-PBR

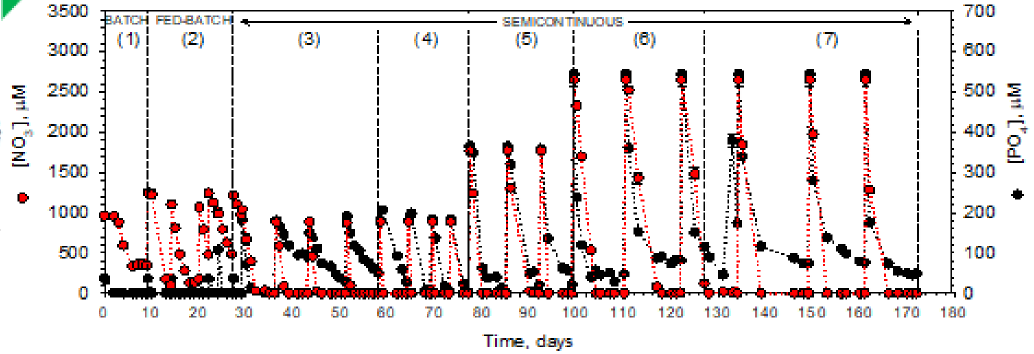
OUTLINE



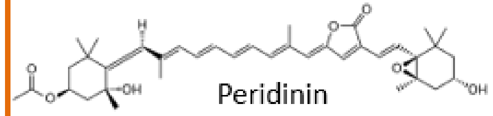


A. Molina-Miras et al., 2018b



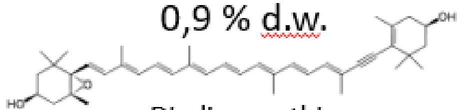


Carotenoids



Peridinin

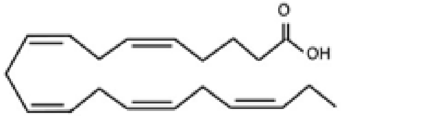
0,9 % d.w.



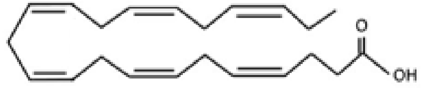
Diadinoxanthin

0,27 % d.w.

PUFAs



EPA 1,69% d.w.

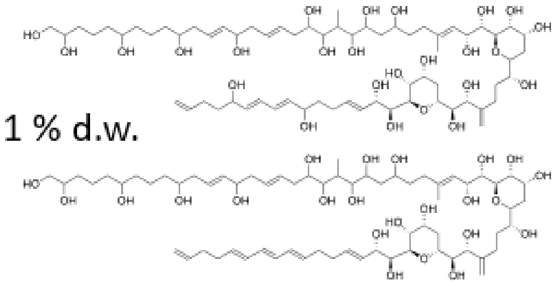


DHA 3,47% d.w.

Bioactive substances

e.g. amphidinols

1 % d.w.



4. Conclusions

OUTLINE

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Conclusions

➢The use of genetic algorithms (**GAs**) has been shown as an **efficient strategy for developing complex culture media and optimization of culture conditions** in bubble columns

➢The use of artificial neural networks (***ANNs***) in **modeling the growth of dinoflagellates** allowed to determine **the relative importance** of **nutrient medium on growth**

➢**Optimization** of small-scale **culture conditions** is necessary to study the growth and production of biocompounds. Due to the **high sensitivity** to hydrodynamic stress of dinoflagellates, it is necessary to pay attention to the **operating conditions of the PBR.**

➢**Water treatment** bioprocesses can be adapted to cultivate dinoflagellates and obtain high added value products.

➢The **scale-up** of bioprocesses based on dinoflagellates **is possible**. **Robust and stable cultures can be achieved..**

XIX FENACAM'23 NATAL/RN-BRASIL 14 a 17 de novembro de 2023

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