Microalgae working for a cleaner planet: waste valorization, resource recovery and circular innovation

Presenters: Luísa Barreira, Davide Liberti

17/04/2025

Luísa Barreira

- Associate Professor – Department of Chemistry and Pharmacy from the Faculty of Sciences and Technology, University of Algarve
- Principal Researcher, Centre of Marine Sciences (CCMAR), co-leader of the Marine Biotechnology Group
- Member of the GreenCoLab – non-profit private organization, a collaborative platform to bridge the transfer of knowledge from academia, industry, stakeholders, and consumers.
- Researcher in microalgae for 16 years

Davide Liberti

- Post-Doc – Centre of Marine Sciences (CCMAR), coordinator of the scientific area of Bioremediation and wastewater treatment from MarBiotech
- PhD in Biotechnology at Università degli studi di Napoli Federico II
- Researcher in microalgae for 6 years

Research lines:

- Microalgal **bioprospection**, culture **scale-up**, and strain **improvement**
- Algae as **functional** food and feed
- Algal **biomedical** applications (nutraceuticals, cosmeceuticals, pharmaceuticals)
- **Bioremediation**, wastewater treatment, and nutrient **recycling** within the concept of circular economy

Microalgae and Cyanobacteria

Photosynthetic microorganisms

- Unicellular or colonial
- Eukaryotic or prokaryotic (cyanobacteria)
- Freshwater or marine species
- Size 0.2 – 200 µm
- Different colours (pigment composition)
- Different shapes

Microalgae Industrial Cultivation

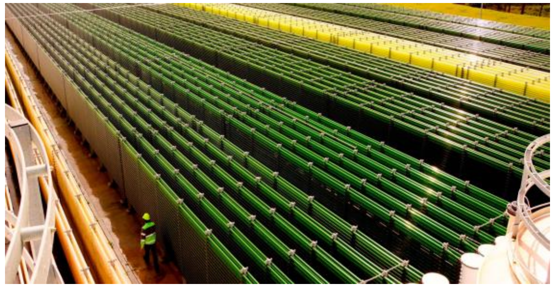
Open systems

Low energy demand
Less control
Possible contaminations



Closed systems

High control
High energy consumption
Very low risk of contamination

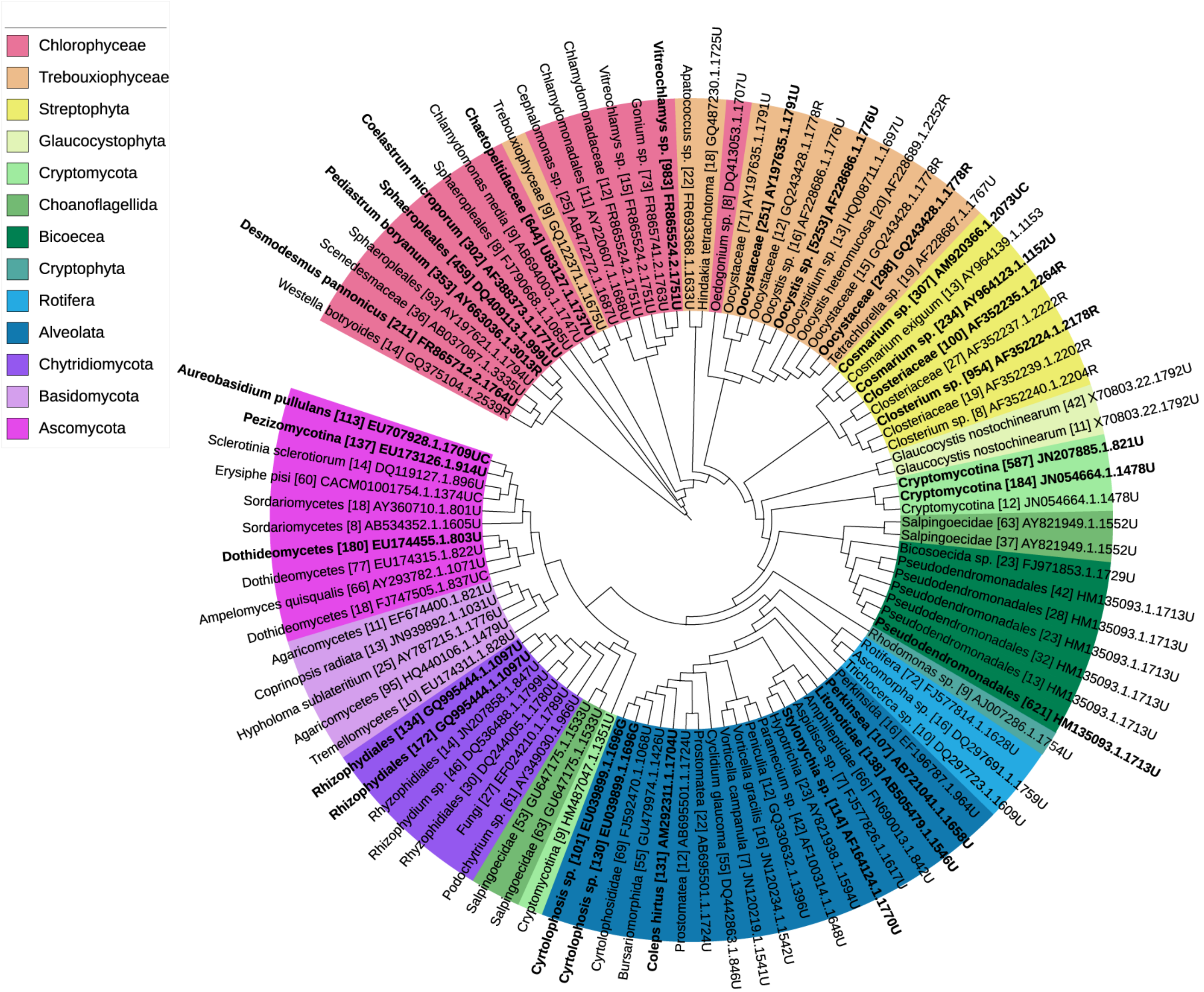


Biodiversity

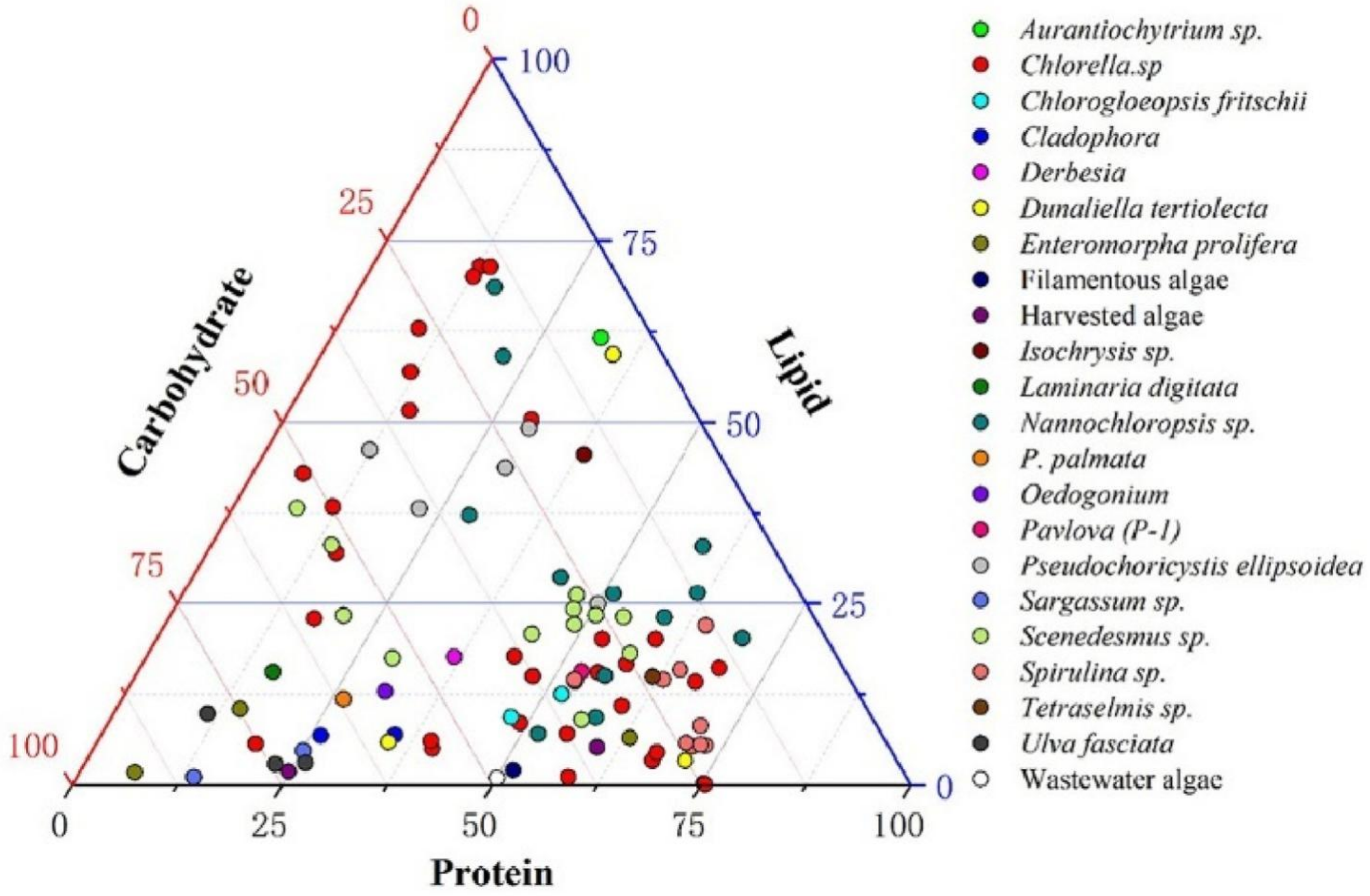
Microalgae have been estimated to include anything between 200,000 to 800,000 species

Less than 50,000 are described

Less than 50 are cultivated at industrial scale

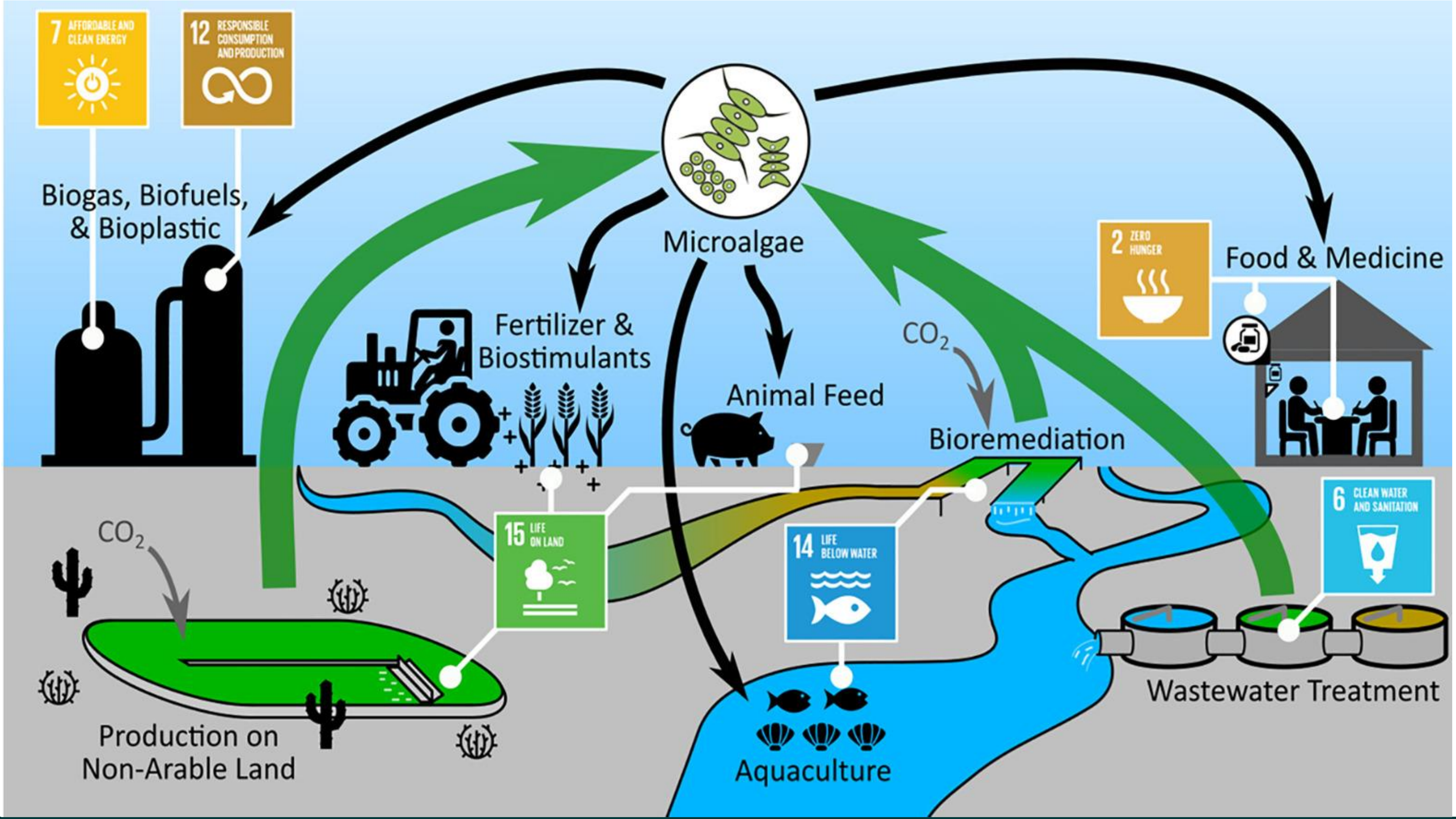


Proximal Composition



Gurreri et al. (2023) Sustainable Chemistry and Pharmacy

Applications

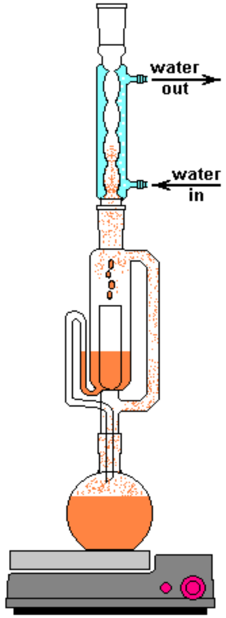


Sutherland et al. (2021) Current Research in Environmental Sustainability

Biofuels production

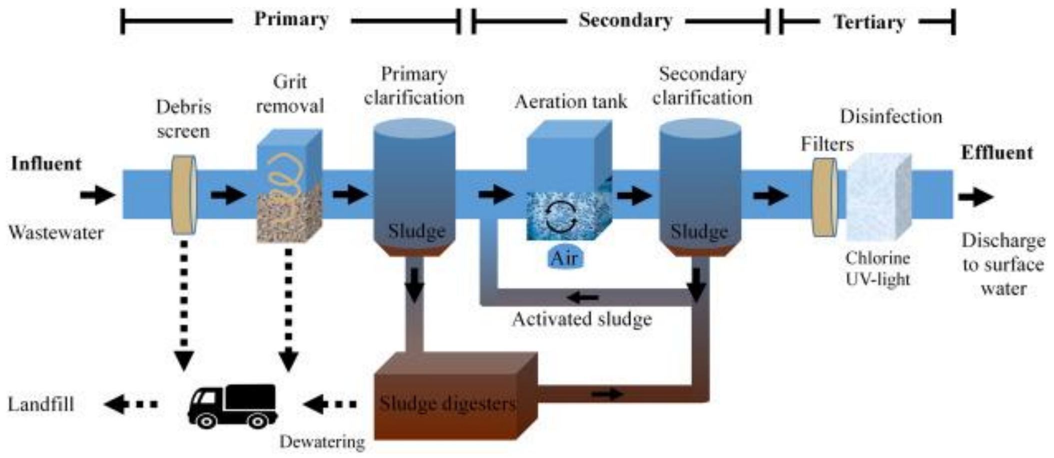
Cultivation

Lipids extraction



Biofuels

Bioremediation



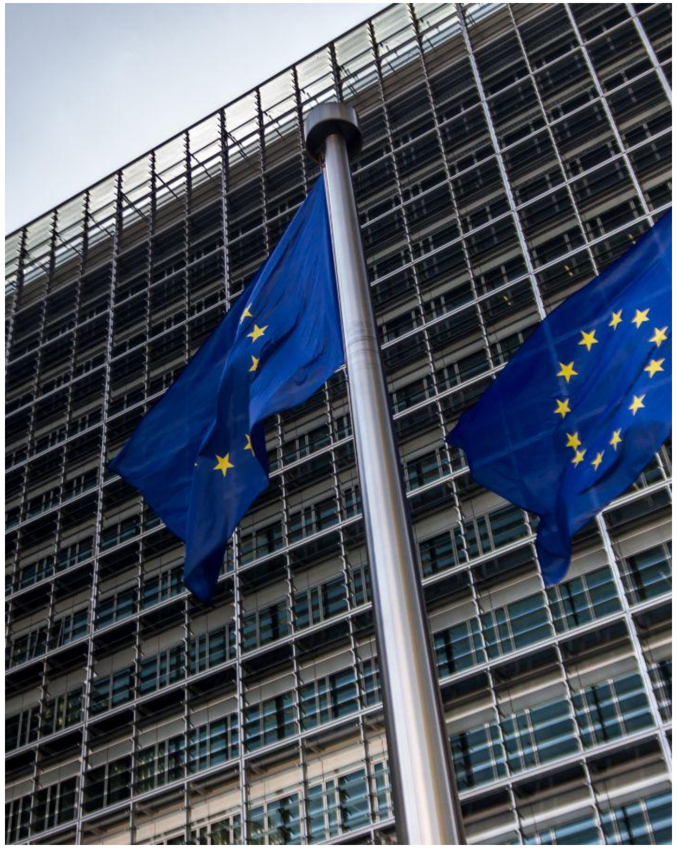
marbiotech marine biotechnology group

CCMAR Centro de Ciências do Mar

RHEMEDiation

European MARINE BOARD Advancing Seas & Ocean Science

Water Framework Directive / Wastewater Treatment Regulation



Regularly updated
Lower limits for Nitrogen and Phosphorous
Higher coverage for smaller towns and rural areas
Extended list of emerging pollutants

https://environment.ec.europa.eu/topics/water/water-framework-directive_en

Official Journal of the European Union — EN — L series

2024/3019 — 12.12.2024

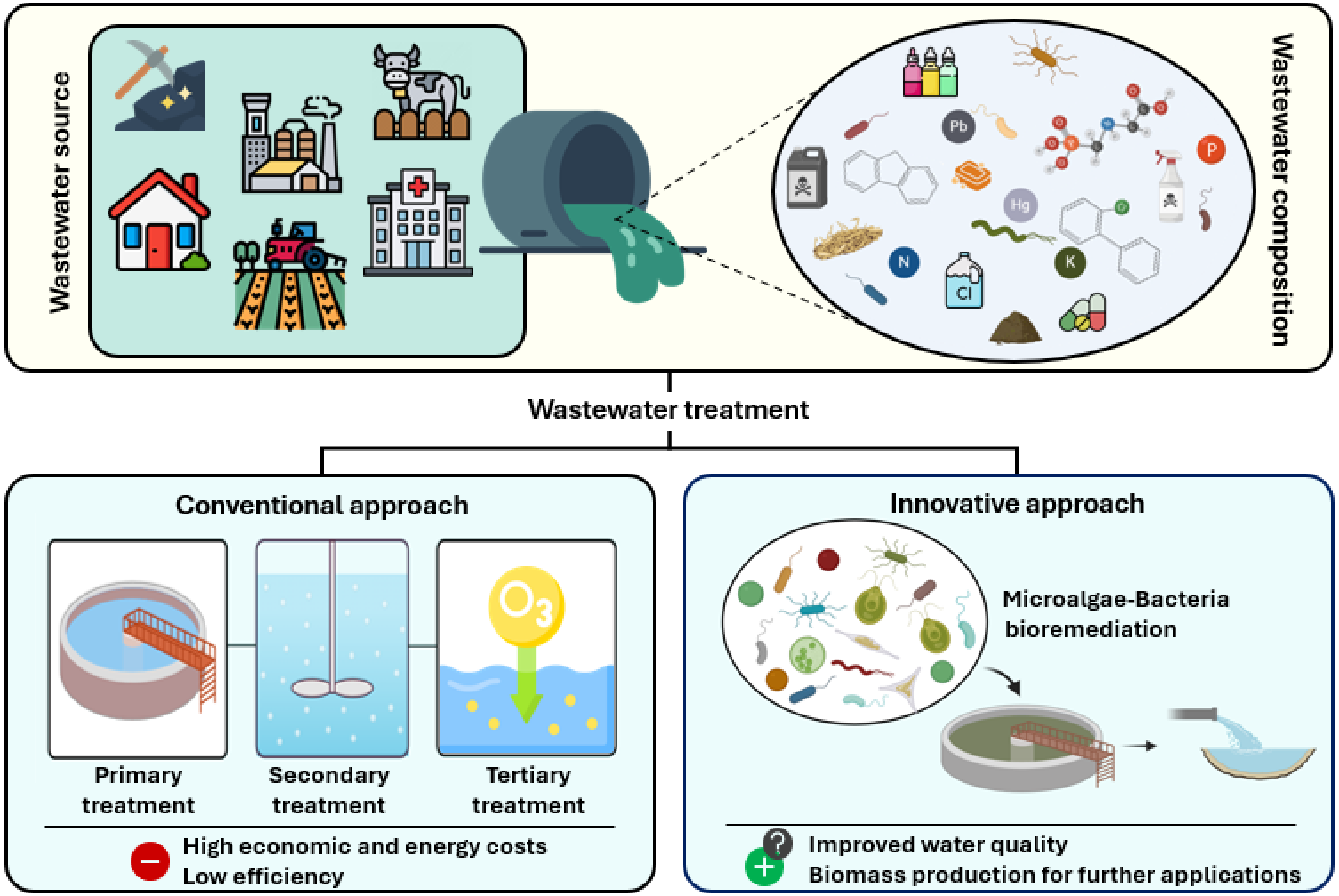
DIRECTIVE (EU) 2024/3019 OF THE EUROPEAN PARLIAMENT AND OF THE COUNCIL

of 27 November 2024

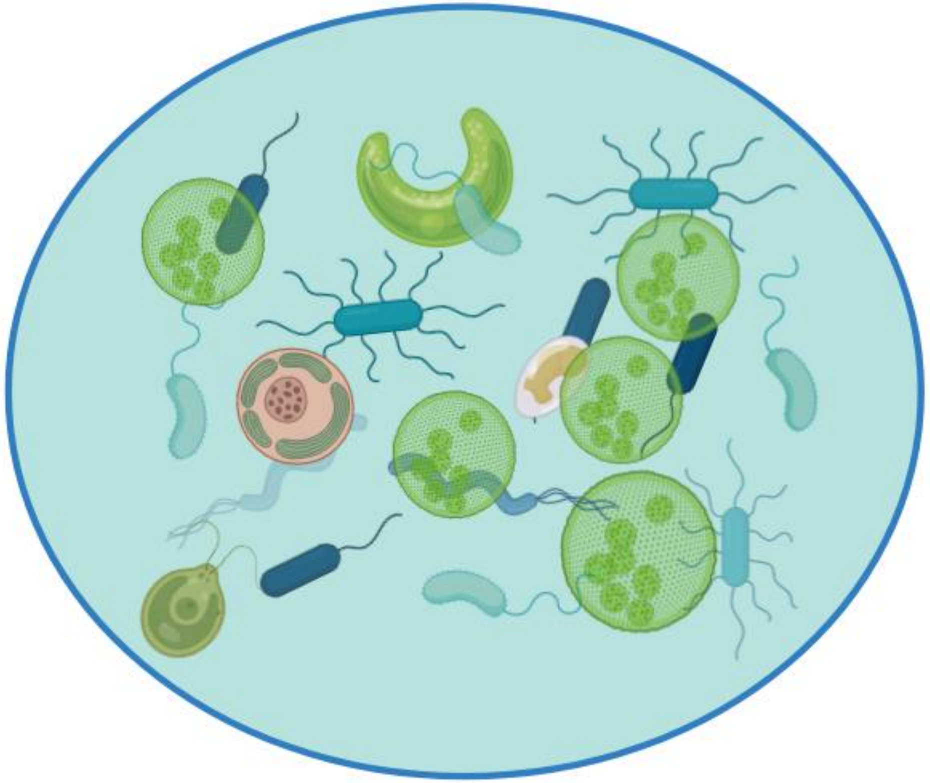
concerning urban wastewater treatment

https://eur-lex.europa.eu/eli/dir/2024/3019/oj/eng

Conventional Wastewater Treatment



Bioremediation



Biotransformation

Bioaccumulation

- Nitrogen
- Phosphorous
- Heavy Metals
- Polychlorinatred Byphenyls (PCBs)
- Polycyclic Aromatic Hydrocarbons (PAHs)
- Pesticides
- PolyFluoroAlkyl Substances (PFAS)
- Pharmaceuticals

Compound	Removal (%)	Species	Installation
PFAS			
PFOA	37	*Synechocystis* sp.	Labscale
PFOS	88	*Synechocystis* sp.	Labscale
Pesticides			
Dimethomorph	24	*Tetradesmus obliquus*	Labscale
	15	*Tetradesmus quadricauda*	Labscale
Isoproturon	54	*Tetradesmus obliquus*	Labscale
	58	*Tetradesmus quadricauda*	Labscale
Pyrimethanil	7.0	*Tetradesmus obliquus*	Labscale
	10	*Tetradesmus quadricauda*	Labscale
Chlorpyrifos	97	*Chlorella* sp. and *Scenedesmus* sp.	Labscale
Oxadiazon	88	*Chlorella* sp. and *Scenedesmus* sp.	Labscale
Cypermethrin	74	*Chlorella* sp. and *Scenedesmus* sp.	Labscale
Heavy metals			
Arsenic	40.7	*Scenedesmus almeriensis*	Labscale
	48	*Ulva lactuca*	Labscale
	59.5	*Ulva Reticulata*	Labscale
Boron	38.6	*Scenedesmus almeriensisis*	Labscale
Cadmium	86	*Phormidium ambiguum*	Labscale
	58	*Desmodesmus* sp. MAS1	Labscale
	17	*Chlorococcum humicola*	Labscale
	75	*Porphyra leucosticta*	Labscale
	95	*Oedogonium westi*	Labscale
Chromium	93.38	*Enteromorpha intestinalis*	Labscale
	66	*Cladophora glomerata*	Labscale
	80	*Pseudochlorella pringsheimii*	Labscale
	93	*Oedogonium westi*	Labscale
	85	*Cystoseira barbata*	Labscale
	85	*Cystoseira crinita*	Labscale
Cobalt	44	*Chlorococcum humicola*	Labscale
Copper	80	*Desmodesmus* sp. AARLG074	Labscale
	88	*Chlorophyceae* spp.	Labscale
	86	*Ulva lactuca*	Labscale
Iron	74.47	*Chlorococcum humicola*	Labscale
Lead	100	*Gelidium amansii*	Labscale
	61-97	*Oedogonium westi*	Labscale
	70	*Phormidium ambiguum*	Labscale
	95	*Porphyra leucosticta*	Labscale
Manganese	99.4	*Chlorella vulgaris*	Labscale
	74	*Ulva lactuca*	Labscale
Mercury	98	*Ulva lactuca*	Labscale
	97	*Phormidium ambiguum*	Labscale
Nikel	59-89	*Oedogonium westi*	Labscale

PCBs			
n.a	*Chlorella pyrenoidosa*	Spiked culture medium	Labscale
n.a			
n.a			
n.a	*Chlamydomonas reinhardtii*	Spiked culture medium	Labscale
n.a			
n.a			
n.a			
n.a			
n.a			
n.a			
n.a			
n.a			
n.a			
n.a			
n.a			
Pharmaceuticals			
99	*Chlorella* sp. and *Scenedesmus* sp.	Urban wastewater	Labscale
99			
not removed			
29.99	*Chlorella sorokiniana*	Spiked culture medium	Labscale
21.58	*Chlorella vulgaris*		
79.09	*Scenedesmus obliquus*		
>99	Natural microalgal consortium	Domestic wastewater	Pilot outdoor
>99			
36-85			
10-69			
33-100			
93			
30-57			
49			
37-53	Natural microalgal consortium	Domestic wastewater	Pilot outdoor
>99	*Chlorella vulgaris*	Aqueous media	Labscale
85.3	Natural microalgal consortium	Domestic wastewater	Pilot outdoor
>91			
>36			
17-53			
not removed			
>99			
51.3			
78			
0.91-100	Natural microalgal consortium	Urban wastewater	Pilot outdoor

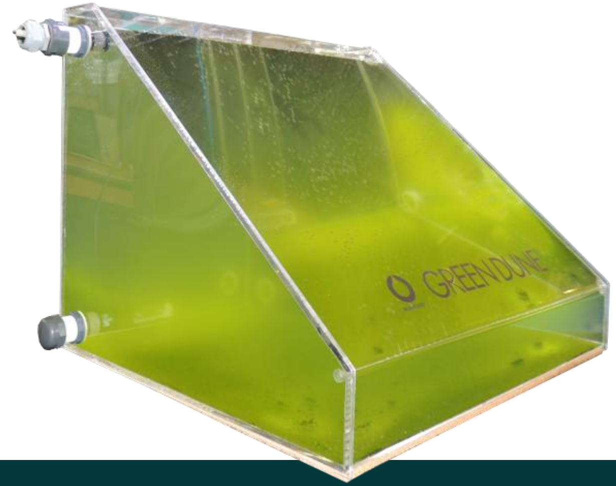
PAHs			
∑PAHs	89-99	*Nannochloropsis oculata*	Labscale
∑PAHs	91-98	*Chlorella vulgaris*	Labscale
Benzo[a]anthracene	68-92	*Selenastrum capricornutum* and *Scenedesmus acutus*	Labscale
Benzo[a]pyrene	62-87		
Phenanthrene	86.64	*Nostoc calciola*	Labscale
	46.67	*Scenedesmus* sp.	
	73.13	*Chlorella* sp.	
	82.7	*Anabaena* sp.	
	77.31	*Leptolyngbya fragilis*	
Pyrene	78.71	*Chlorela* sp.	Labscale
	95	*Oscillatoria* sp.	
Benzo[a]anthracene	77.7-90.9	*Selenastrum capricornutum*	Labscale

Wastewater Treatment With Microalgae

Advantages: Sustainable system | Viable costs | Biomass production | Less CO_2 and GHG emissions

High land requirements in standard cultivation systems

GREENDUNE PHOTOBIOREACTORS



BLUEMATER
ECO-EFFICIENT SOLUTIONS

- ✓ High volume/area ratio: 480 L m^{-2}
- ✓ Modular systems
- ✓ Connected in sequence or in parallel to achieve the total volume required

The aim of this study was to evaluate microalgae biomass production, and tertiary treatment efficiency of urban wastewater using natural microalgal consortium in pilot GreenDune PBRs

Wastewater Treatment With Microalgae

green treat

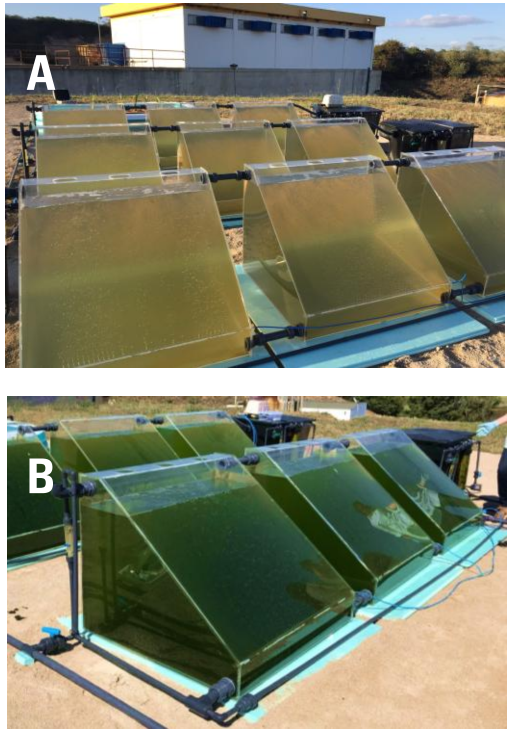


Figure 2 Natural Blooms development in GreenDune PBRs
A) Stabilization phase and **B**) During the experiments

Experimental set-up with 3 sequential modules with a total working volume of 1440L installed at wastewater treatment plant at Algarve, Portugal

DAILY ANALYSIS

Cell growth control

- ✓ pH measurements
- ✓ Optical density (750 nm)

Treatment efficiency

- ✓ total-N
- ✓ $N\text{-}NH_4$,
- ✓ $N\text{-}NO_3$

OUTDOOR CONDITIONS

Continuous operational mode

- ✓ Spring (June 2020) – HRT 24 h and 12 h
- ✓ Summer (August 2020) – HRT 24 h and 48 h

Natural Bloom Culture

- ✓ 7 days of stabilization
- ✓ 12 days of culture

marbiotech marine biotechnology group

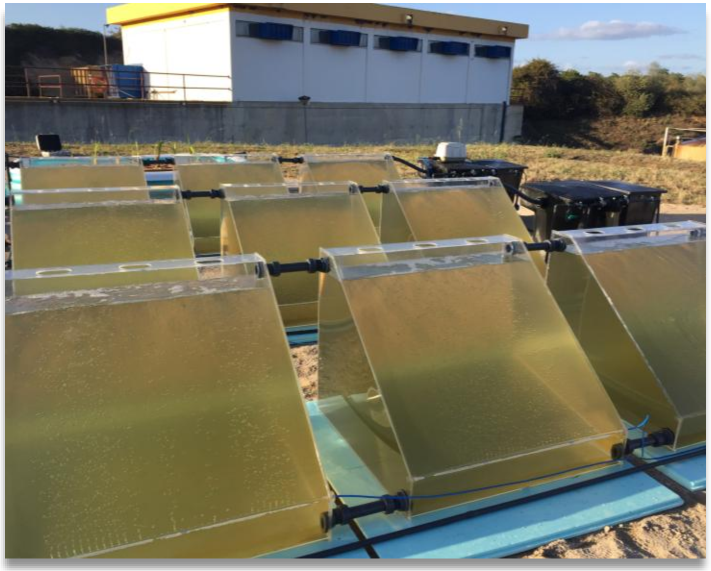
CCMAR Centro de Ciências do Mar

RHEMEDiation

European MARINE BOARD Advancing Seas & Ocean Science

green treat

Wastewater Treatment With Microalgae



HRT – 24h, 48h (Winter, Summer, Autumn)
HRT – 12h, 24h (Spring)

Water reuse for for seed production or irrigation of naturally restricted use areas

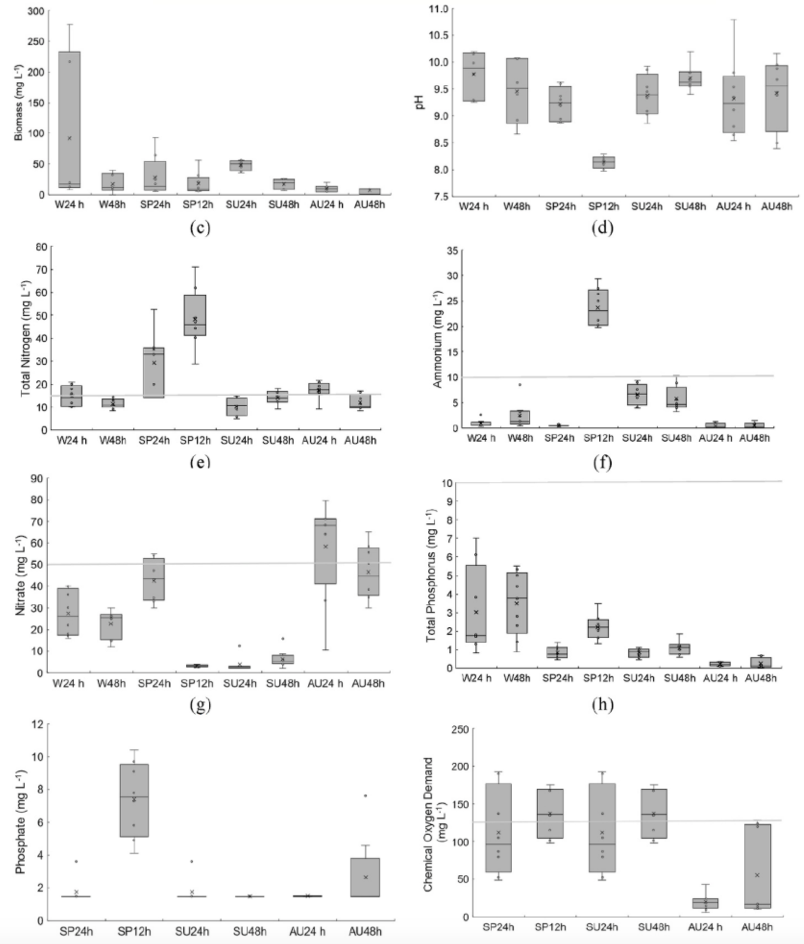
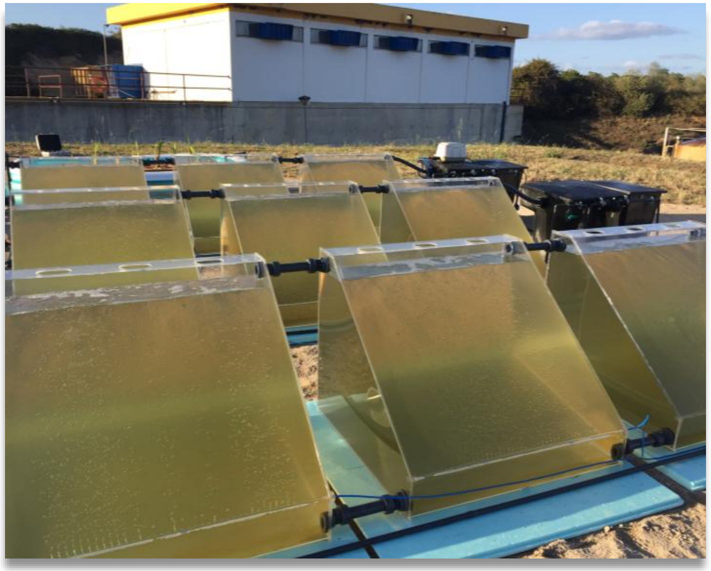


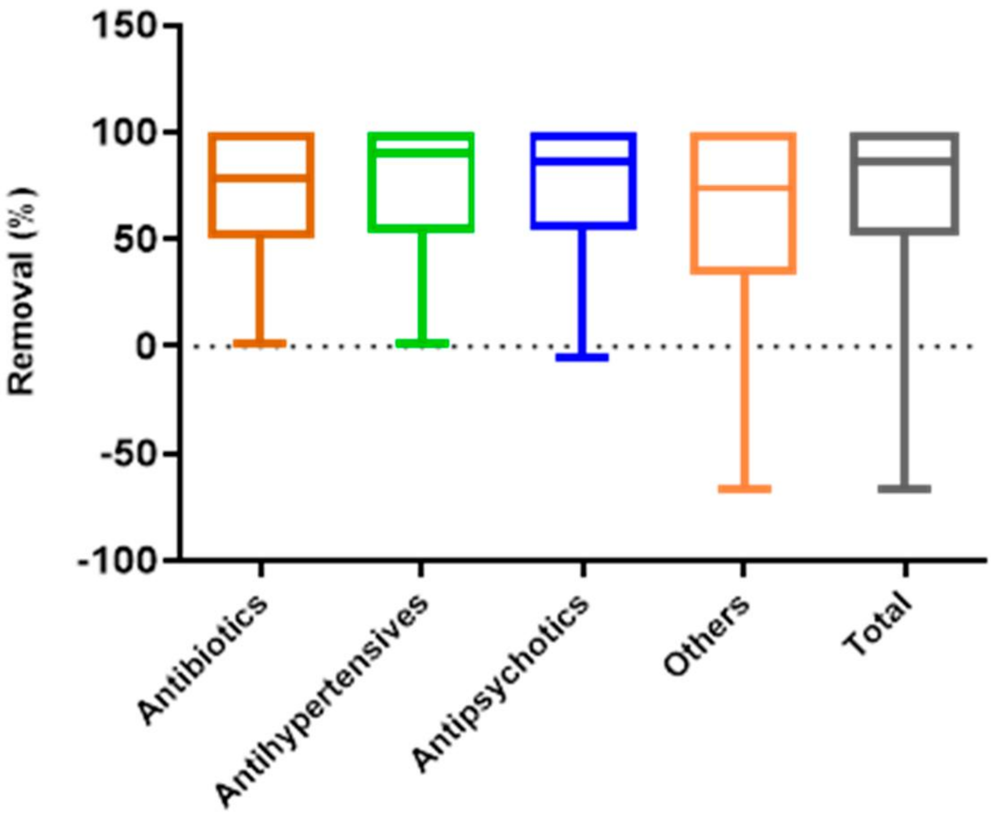
Fig. 2. Biomass concentration (mg L^{-1}) (a), pH variation (b), and water quality parameters in the PBRs effluent (treated wastewater) (c) TN, (d) NH_4^+, (e) NO_3^-, (f) TP, (g) PO_4^{3-} and (h) COD in the different seasons and hydraulic retention times: winter 24h (W24h), winter 48h (W48h), spring 24h (SP24h), spring 12h (SP12h), summer 24h (SU24h), summer 48h (SU48h), autumn 24h (AU24h) and autumn 48h (AU48h). In these diagrams, the x represents the average value and the horizontal dash the median of the nutrient's concentration. The box limits are defined by the lower and upper quartiles of the data and the whiskers are the minimum and maximum values of the data set. The horizontal lines across the charts represent the legal limit for that nutrient in the discharged treated wastewater.

Morais et al. (2020) J Cleaner Production

Pereira et al. (2023) Applied Sciences

Wastewater Treatment With Microalgae





HRT – 24h, 48h (Winter, Summer, Autumn)
HRT – 12h, 24h (Spring)

Water reuse for for seed production or irrigation of naturally restricted use areas

Morais et al. (2020) J Cleaner Production

Pereira et al. (2023) Applied Sciences

marbiotech marine biotechnology group

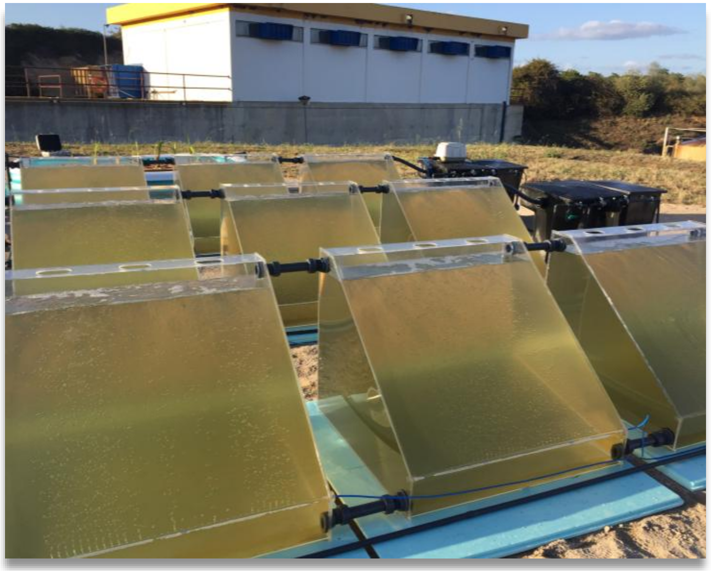
CCMAR Centro de Ciências do Mar

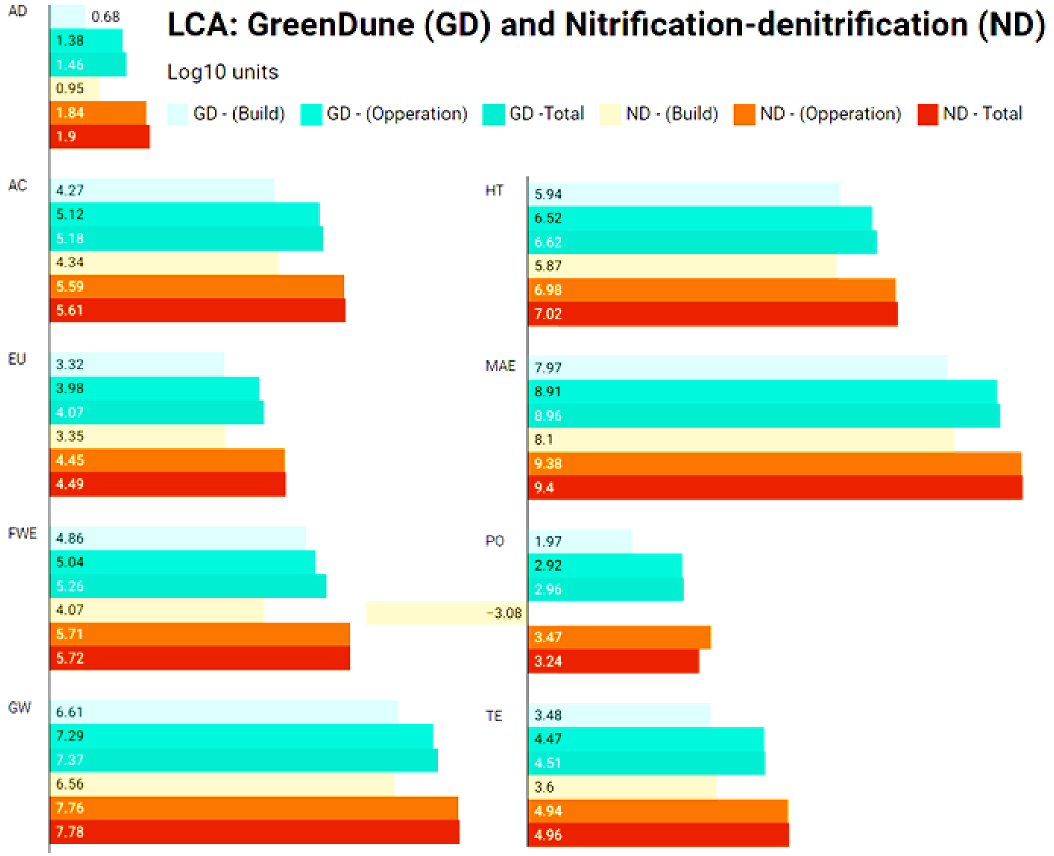
RHEMEDiation

European MARINE BOARD Advancing Seas & Ocean Science

greentreat

Wastewater Treatment With Microalgae





HRT – 24h, 48h (Winter, Summer, Autumn)
HRT – 12h, 24h (Spring)

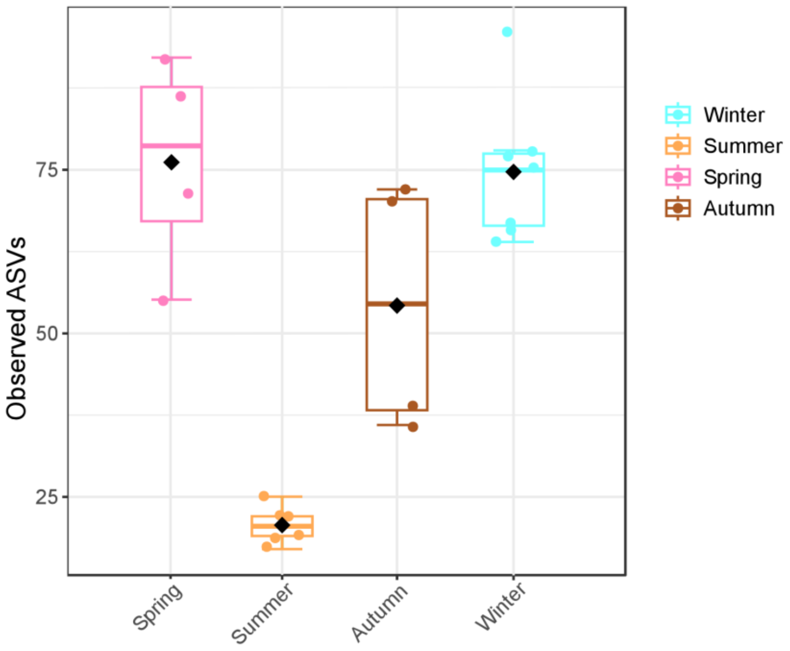
Water reuse for for seed production or irrigation of naturally restricted use areas

Morais et al. (2020) J Cleaner Production

Pereira et al. (2023) Applied Sciences

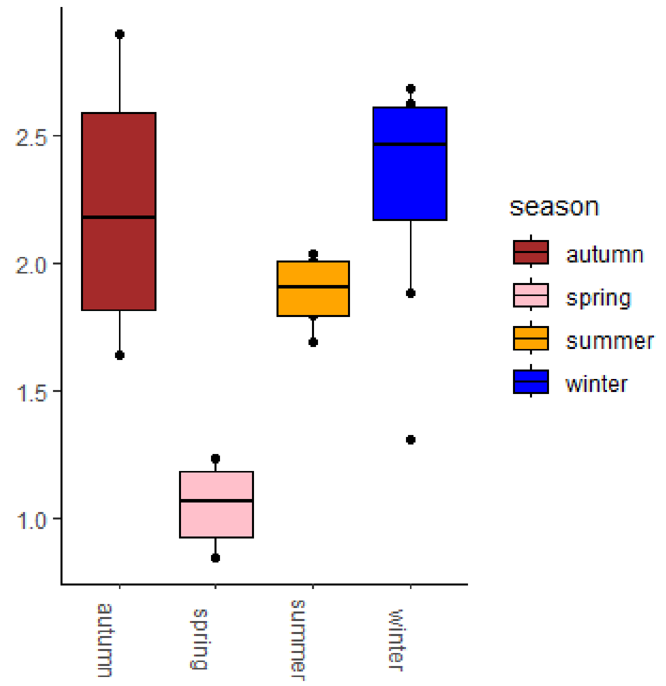
Wastewater Treatment With Microalgae: Green Treat

α-diversity

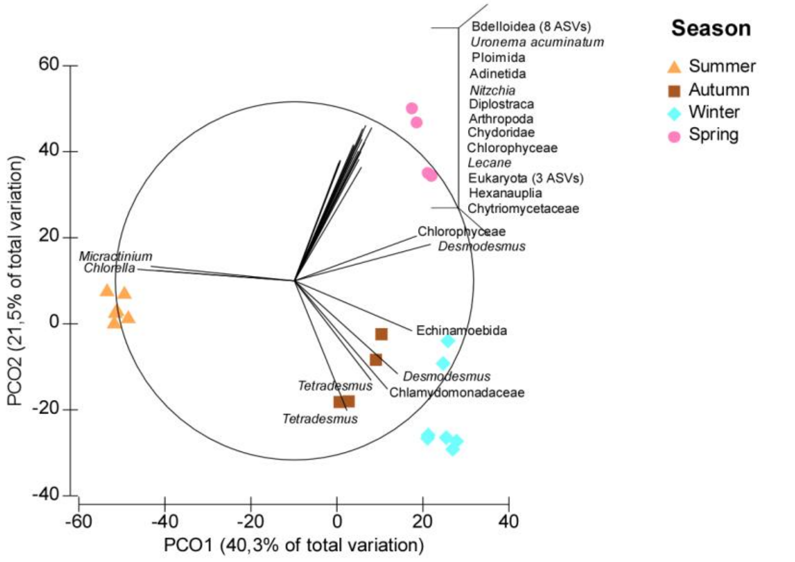


Species richness

Shannon



Species richness and evenness



Spring: dominated by rotifers!

Autum and Winter: *Tetradesmus* sp.

Summer: *Chlorella* sp.

Biodiversity changes with seasons!

Wastewater Treatment With Microalgae: Green Treat

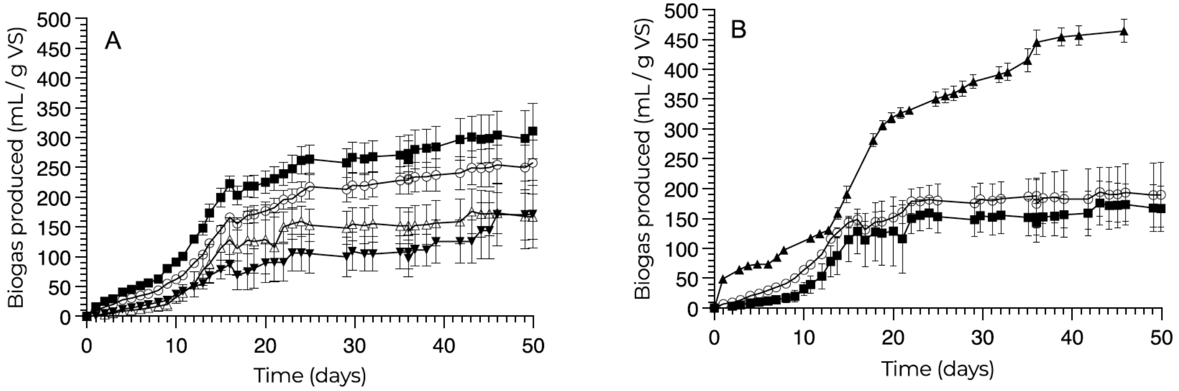


Figure 2. Accumulated biogas production after 50 d: (**A**) different seasons (○) summer (▼) autumn (■) spring (△) winter and (**B**) winter season with different HRT (■) 24 h and (○) 48 h and (▲) *Skeletonema*monoculture. The data plotted are the differences between the means of the digested sample and the mean of the inoculum control (all *n*= 3). Error bars represent standard deviation.

Table 2. Maximum biogas and methane production (mL/g volatile solids) using non-hydrolyzed (NH) and hydrolyzed (H) biomass.

Product	Summer	Autumn	Spring	Winter24	Winter48	*Skeletonema*
Biogas NH	258 ±38 b,c	172 ±57 c	311 ±47 b	167 ±37 c	190 ±55 b,c	464 ±19 a
Methane NH	211 ±97 *,#	155 ±32 *,#	252 ±36 *,#	149 ±24 *,#	135 ±63 *,#	-
Methane H	189 ±8 #	81 ±11 #	239 ±12 #	191 ±29 #	137 ±21 #	-

The reported results are the difference between the means of the digested sample and the mean of the inoculum control (all *n*= 3) ±the respective standard deviation. The same letters ([a,b,c] used to compare biogas production), or symbols (* used to compare methane production between all non-hydrolyzed biomass conditions; # used to compare methane production between non-hydrolyzed and hydrolyzed conditions of the same season) indicate no statistical difference between tested conditions.

Biogas production

Higher biogas production and methane yield in the Spring

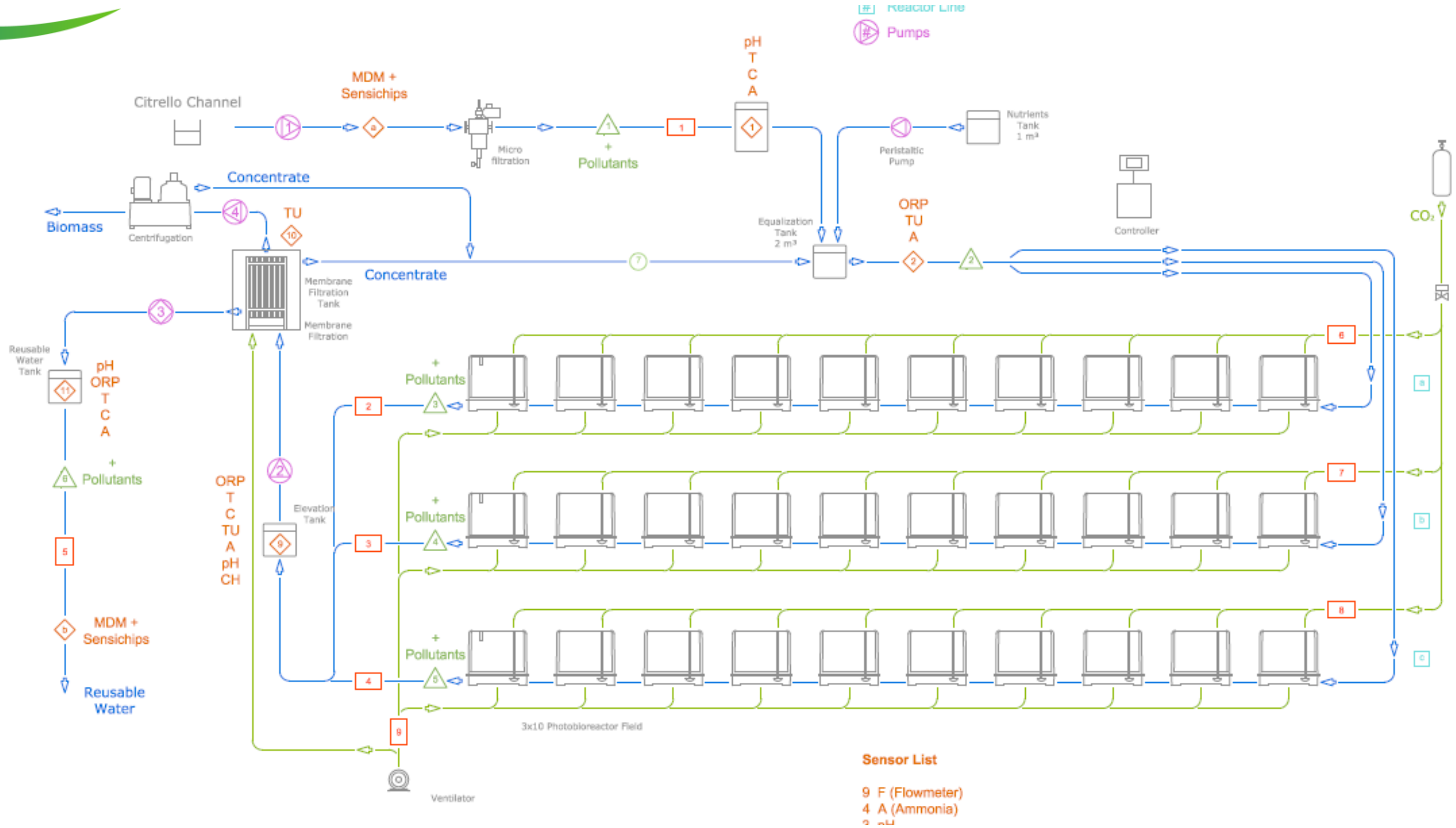
Lower yields than with monoalgal cultures

Low intrinsic biodegradability due to the prevalence of predators?

Case study:

RHE-MEDiation

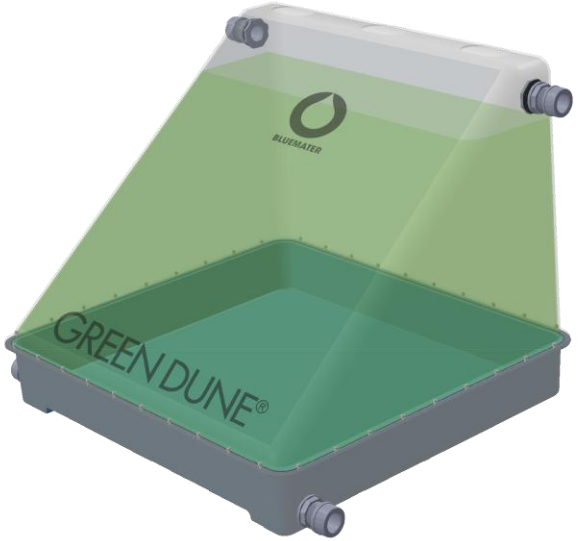
Responsive hub for long term governance to destress the Mediterranean Sea from chemical pollution (RHE-MEDiation)



CCMAR Centro de Ciências do Mar
European Marine Board Advancing Seas & Ocean Science
hcmr ΕΛΚΕΘΕ
BLUEMATER
RINA
TÜBİTAK MAM
EYDAP
sensichips
MDM
YURT MÜHENDİSLİK
Consiglio Nazionale delle Ricerche

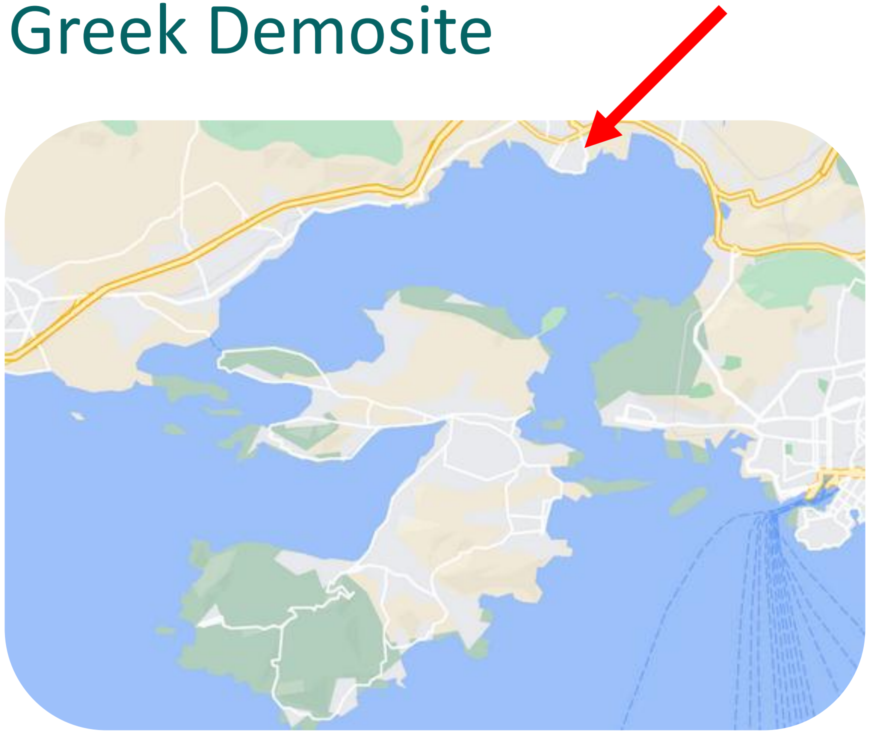
EU MISSIONS RESTORE OUR OCEAN & WATERS

Case study: RHE-MEDiation Project



"Emerging pollutants are defined as compounds that are not currently covered by existing water-quality regulations, have not been studied before, and are thought to be potential threats to environmental ecosystems and [human health](#) and safety"
Farré et al. 2008

Greek Demosite



Located in Greece, Elefsina, and its gulf, is a suburban city from Athens metropolitan area. It is situated in the Thriasio Plain and it is a major industrial centre, with the largest oil refinery in Greece

Taking into account previous collected data, the bioreactors will be placed after the secondary wastewater treatment, to remove excess nutrients and pollutants not removed by the currently implemented system.

Turkish Demosite

Located in Turkey, Izmit Bay is a semi-closed embayment situated in the Marmara Sea. Residential and industrial areas in the region have increased threefold since 1980s, thus increasing the pollution.

In Dilovasi, the bioreactors will befed with primarily treated wastewater, therefore conducting most of the treatment, to demonstrate their versatility.

Case study: RHE-MEDiation Project

The aim is to prevent further accumulation of pollutants in the seawater of Mare Piccolo, pumping water from Galeso River. Mare Piccolo is very important for muscles aquaculture, a very well known specialty from Taranto.

Located in south Italy, Mare piccolo is a bay surrounded by Taranto city. In the past, it has been overloaded with pollutants from several sources, ILVA above all.

List of major contaminants deemed to be removed from EU waters and are in line with the RHE-MEDiation GA, based on the Directive 2013/39/EU (WFD) and the Proposal (for a Directive of the EU and of the Council) amending Directives 2000/60/EC, 2006/118/EC and 2008/105/EC. In blue: compounds common within the WFD and its proposed amendment.

Pesticides	PFAS/PFOS	Pharmaceuticals	Metals	PAHs	PCBs
Aldrin	Perfluorohexane sulfonic acid (PFHxS)	17 alpha-ethinylestradiol	Cd	Anthracene	PCB 77 (Dioxin-like)
Dieldrin	Perfluorononanoic acid (PFNA)	17 beta-estradiol	Pb	Naphthalene	PCB 81 (Dioxin-like)
Endrin	Perfluorobutane sulfonic acid (PFBS)	Azythromycin	Hg	Fluoranthene	PCB 105 (Dioxin-like)
Isodrin	Perfluorohexanoic acid (PFHxA)	Erythromycin	Ni	Benzo[a]pyrene	PCB 114 (Dioxin-like)
Atrazine	Perfluorobutanoic acid (PFBA)	Diclofenac	Ag	Benzo[b]fluoranthene	PCB 118 (Dioxin-like)
Chlorpyrifos	Perfluoropentanoic acid (PFPeA)	Ibuprofen		Benzo[k]fluoranthene	PCB 123 (Dioxin-like)
DDTs	Perfluoropentane sulfonic acid (PFPeS)	Carbamazepine		Benzo[g,h,i]perylene	PCB 126 (Dioxin-like)
Trifluralin	Perfluorodecanoic acid (PFDA)	Clarithromycin		Indeno[1,2,3-cd]pyrene	PCB 156 (Dioxin-like)
Clothianidin	Perfluorododecanoic acid (PFDoDA)	Estrone		Chrysene	PCB 157 (Dioxin-like)
Diuron	Perfluoroundecanoic acid (PFUnDA)			Benzo[a]anthracene	PCB 167 (Dioxin-like)
Endosulfan	Perfluoroheptanoic acid (PFHpA)			Dibenz[a,h]anthracene	PCB 169 (Dioxin-like)
Hexachlorobenzene	Perfluorotridecanoic acid (PFTrDA)				PCB 189 (Dioxin-like)
Hexachlorocyclohexane	Perfluoroheptane sulfonic acid (PFHpS)				
Deltamethrin	Perfluorodecane sulfonic acid (PFDS)				
Esfenvalerate	Perfluorotetradecanoic acid (PFTeDA)				
Isoproturon	Perfluorohexadecanoic acid (PFHxDA)				
Pentachlorophenol	Perfluorooctadecanoic acid (PFODA)				
Dicofol	Ammonium perfluoro (2-methyl-3-oxahexanoate) (HFPO-DA or Gen X)				
Aclonifen	Propanoic Acid / Ammonium 2,2,3-trifluoro-3-(1,1,2,2,3,3-hexafluoro-3-(trifluoromethoxy)propoxy)propanoate (ADONA)				
Bifenox	2- (Perfluorohexyl)ethyl alcohol (6:2 FTOH)				
Dichlorvos	2-(Perfluorooctyl)ethanol (8:2 FTOH)				
Heptachlor/ Heptachlor epoxide	Acetic acid / 2,2-difluoro-2-((2,2,4,5-tetrafluoro-5-(trifluoromethoxy)-1,3-dioxolan-4-yl)oxy)-(C6O4)				
Terbutrin	Perfluorooctanoic acid (PFOA)				
Acetamiprid	Perfluorooctane sulfonic acid (PFOS)				
Bifenthrin					
Glyphosate					
Imidacloprid					
Nicosulfuron					

marbiotech marine biotechnology group

CCMAR Centro de Ciências do Mar

RHEMEDiation

European MARINE BOARD Advancing Seas & Ocean Science

Greek Demo Site

EU MISSIONS RESTORE OUR OCEAN & WATERS

Greek Demo Site

Reactors: 30 units
Total volume: 15 m^3
Consortium developing time: 10 days
Hydraulic Retention Time: 24 hours
Sensors: 6

Analyzed parameters
pH
Redox potential
Ammonium concentration
Turbidity
Conductivity
Chlorophyll content

Preliminary results

Starting point

After one month

Turkish Demo Site

marbiotech
marine biotechnology group

CCMAR
Centro de Ciências do Mar

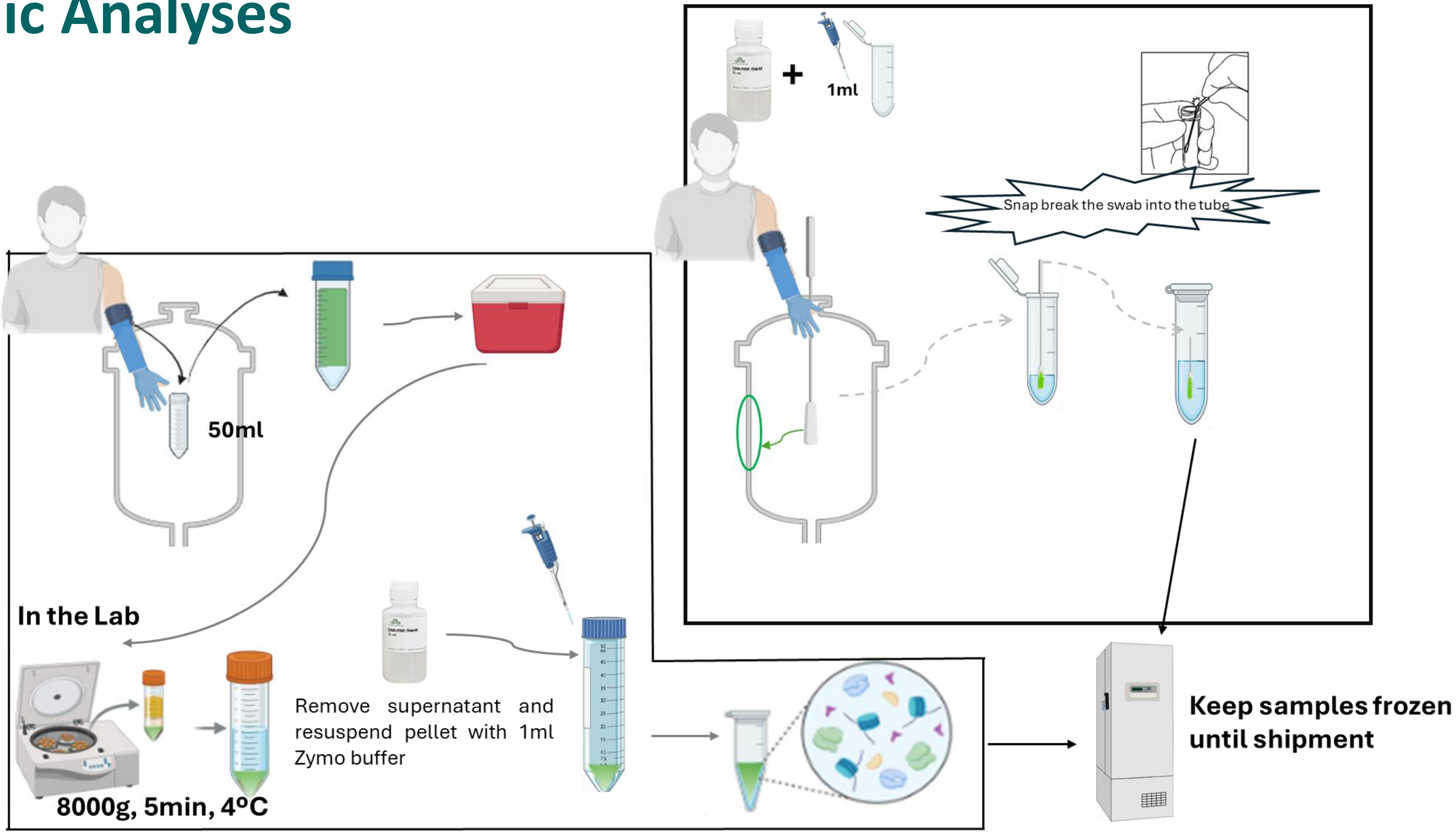
RHEMEDiation

European
MARINE BOARD
Advancing Seas & Ocean Science

Turkish Demo Site

EU MISSIONS
RESTORE OUR OCEAN & WATERS

Metagenomic Analyses



marbiotech marine biotechnology group

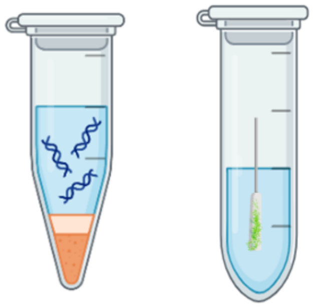
CCMAR Centro de Ciências do Mar

RHEMEDiation

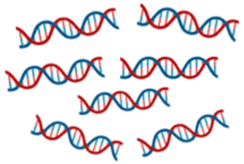
European MARINE BOARD Advancing Seas & Ocean Science

Metagenomic Analyses

Lab Work



DNA extraction



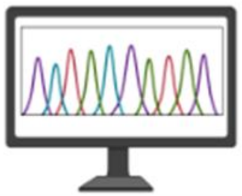
Outsourced

16S Bacteria

18S Eukaryotes

Illumina MiSeq

Data Analysis

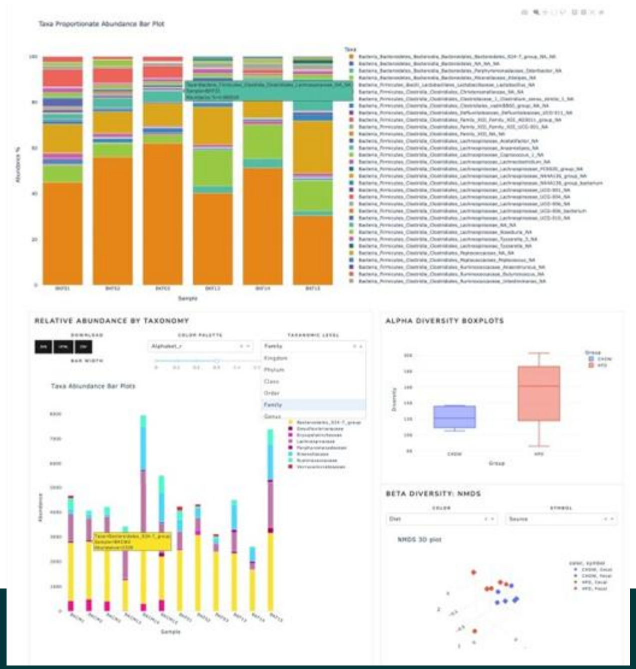


qiime2

DADA2 Amplicon Sequencing. Exactly. Version 1.18

silva ngs

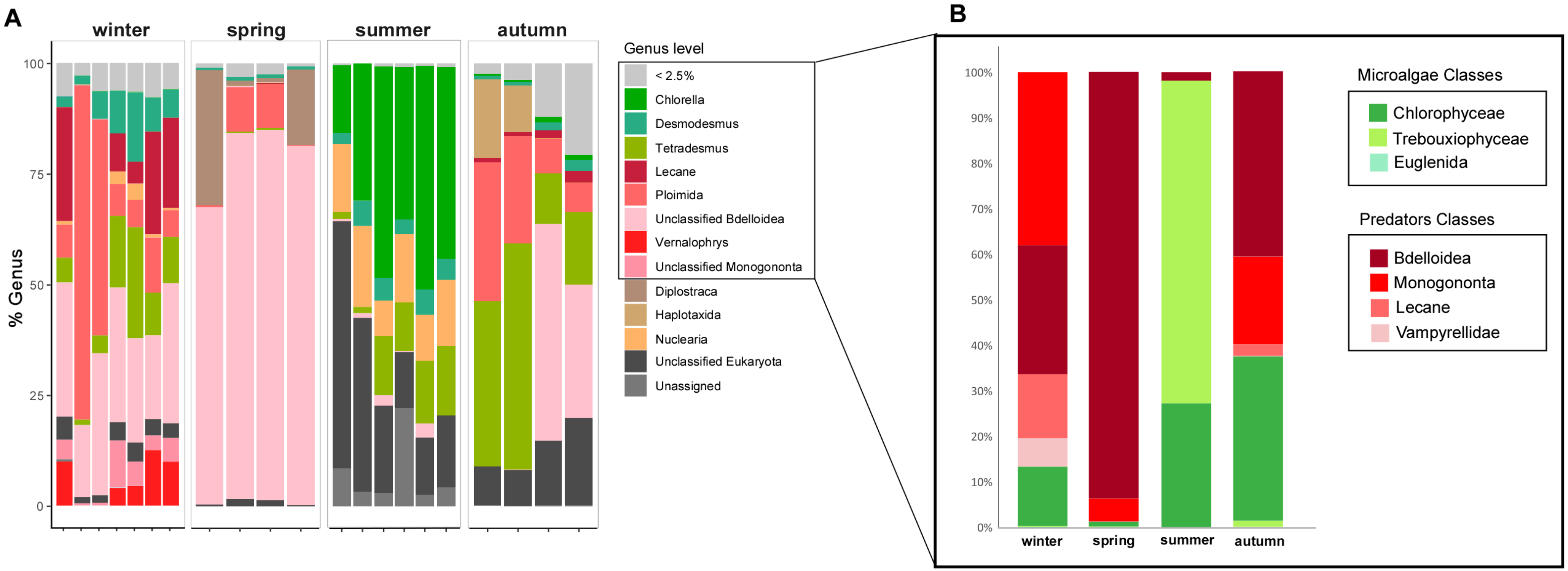
Data visualization



EU MISSIONS RESTORE OUR OCEAN & WATERS

Metagenomic Analyses

Community characterization – Abundance Plots

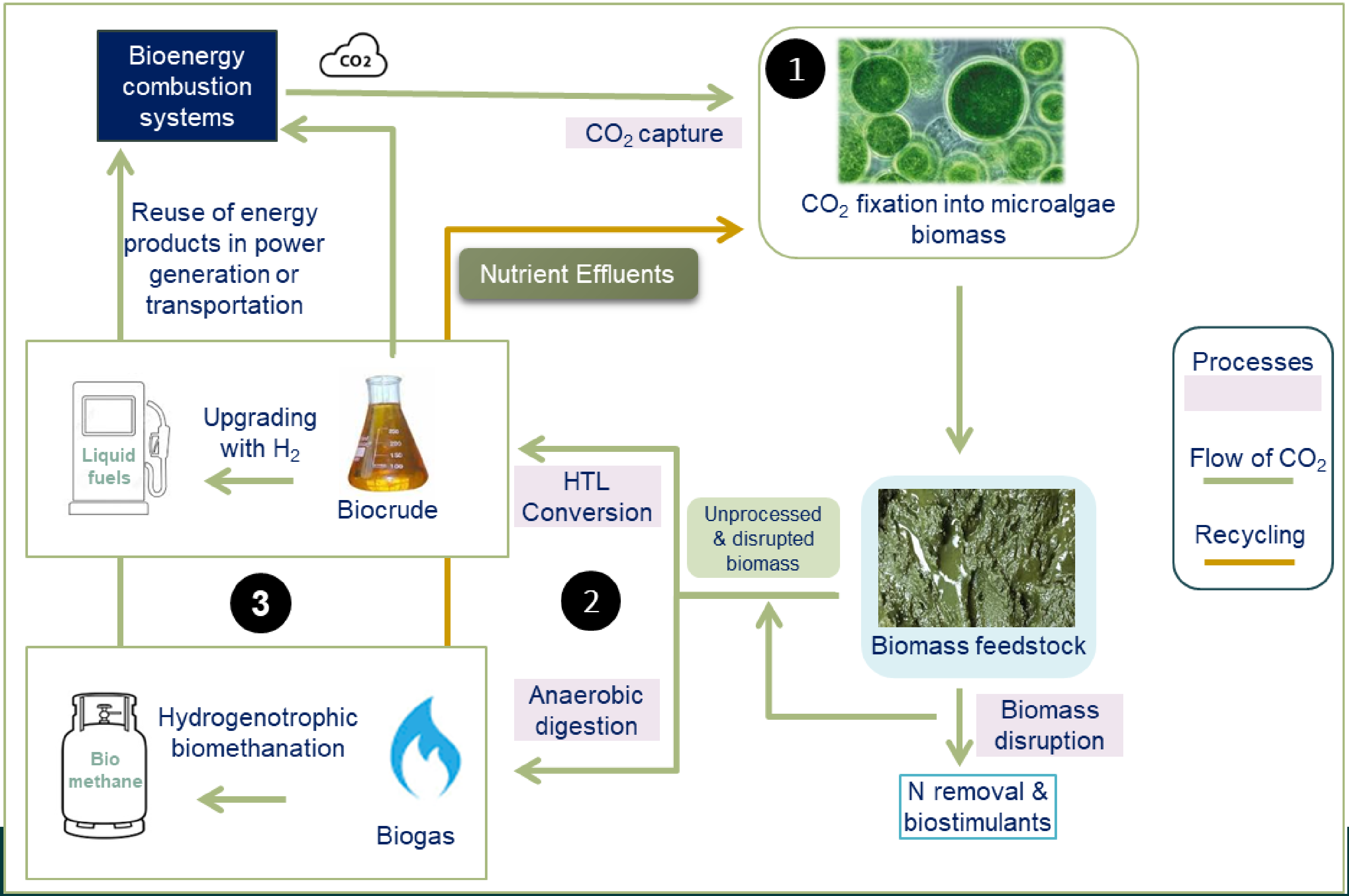


EU MISSIONS
RESTORE OUR OCEAN & WATERS

Case study: COSEC

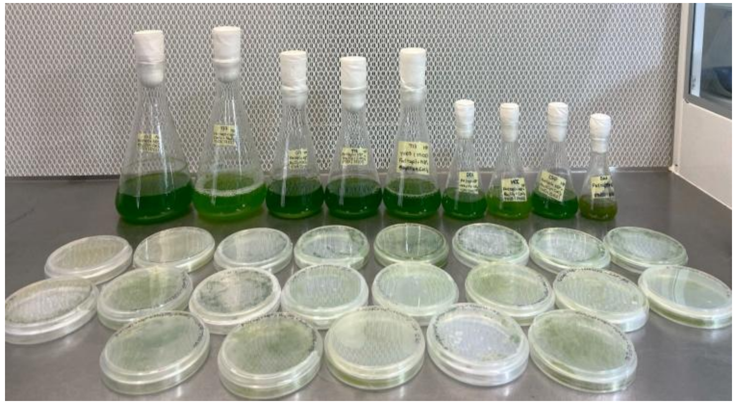
Project

Biogenic CO_2 capture into Sustainable Energy Carriers - A novel photosynthetic and hydrogenotrophic CO_2 fixation combined with waste nutrient upcycling for production of carbon negative energy carriers.



Case study: COSEC Project
Work Pipeline – Strain selection

Task 2.1. Optimise fixation of biogenic CO_2 and residual nutrients into microalgae biomass
Task 3.1: Strain development for improved resilience to flue gases and production of lipid rich biomass



Textile wastewater

Switzerland

Greenhouse wastewater

Urban wastewater

Case study: COSEC Project

Work Pipeline – Strain improvement

1

Determine the **lethal** and **sub-lethal** concentration for **metabolic inhibitors**

→

Random Mutagenesis
EMS (Ethyl methanesulfonate)

→

Cell viability

2

Random Mutagenesis

→

Apply the screening on metabolic inhibitors

→

Resistant strains isolation and phenotypic stability

→

Validation of improved traits

1. Flow Cytometry
2. Bligh and Dyer

Case study: COSEC Project
Work Pipeline – Strain improvement

1

Determine the **lethal** and **sub-lethal** concentration for **metabolic inhibitors**

→

Random Mutagenesis
EMS (Ethyl methanesulfonate)

→

Cell viability

2

Random Mutagenesis

→

Apply the screening on metabolic inhibitors

→

Resistant strains isolation and phenotypic stability

→

Validation of improved traits
1. Flow Cytometry
2. Bligh and Dyer

Final Considerations

- **Versatile Applications**: Microalgae have potential in various sectors — from biofuels to nutraceuticals and animal feed
- **Sustainability Challenge**: Large-scale production requires improvements in economic, energetic, and environmental sustainability
- **Wastewater as a Resource**: Wastewaters are nutrient-rich (N and P), making them ideal for microalgal growth and offering a low-cost, eco-friendly cultivation method
- **Biorefinery Approach**: Coupling microalgae cultivation with wastewater treatment enables contaminant removal while producing valuable biomass
- **Environmental Benefits**: This approach mitigates environmental impacts from synthetic media and traditional effluent treatments
- **Need for Research & Guidelines**: Few large-scale studies exist; fundamental design and operational guidelines are still lacking
- **Technology Readiness Gap**: Advancing from TRL 3 to TRL 9 is essential for commercial deployment and meeting real-world demands

Conclusions

- The use of microalgae in wastewater treatment can be economically viable, with positive energy balances
- More pilot or demonstration-scale studies are needed to better assess real process costs
- Natural microalgal consortia may be easier to manage than specific strains due to greater adaptability
- The resulting biomass can be valorised as: biofuels, biostimulants / biofertilizers, and animal feed
- It is essential to assess the biomass's chemical composition and presence of toxic compounds before defining its application
- **Main limitation** of microalgae-based systems: large area requirement due to light dependence and long hydraulic retention times
- **Solutions under development**:
    - Vertical systems
    - Reactors with higher volume-to-area ratios
- **Advantages over conventional treatments**:
    - Efficient removal of emerging pollutants
    - Reduction of environmental impacts and treatment costs
- Promising application in rural areas or developing countries, where land availability is higher and treatment systems are often lacking

Thank you!

lbarreir@ualg.pt

dliberti@ualg.pt