



**SWRI**  
Soil and Water Resources Institute



10th International Conference on Sustainable Solid Waste Management Chania, Greece,  
21 - 24 JUNE 2023

## Food industry wastewater valorization for microalgae growth and the use of microplates as a microreactor platform

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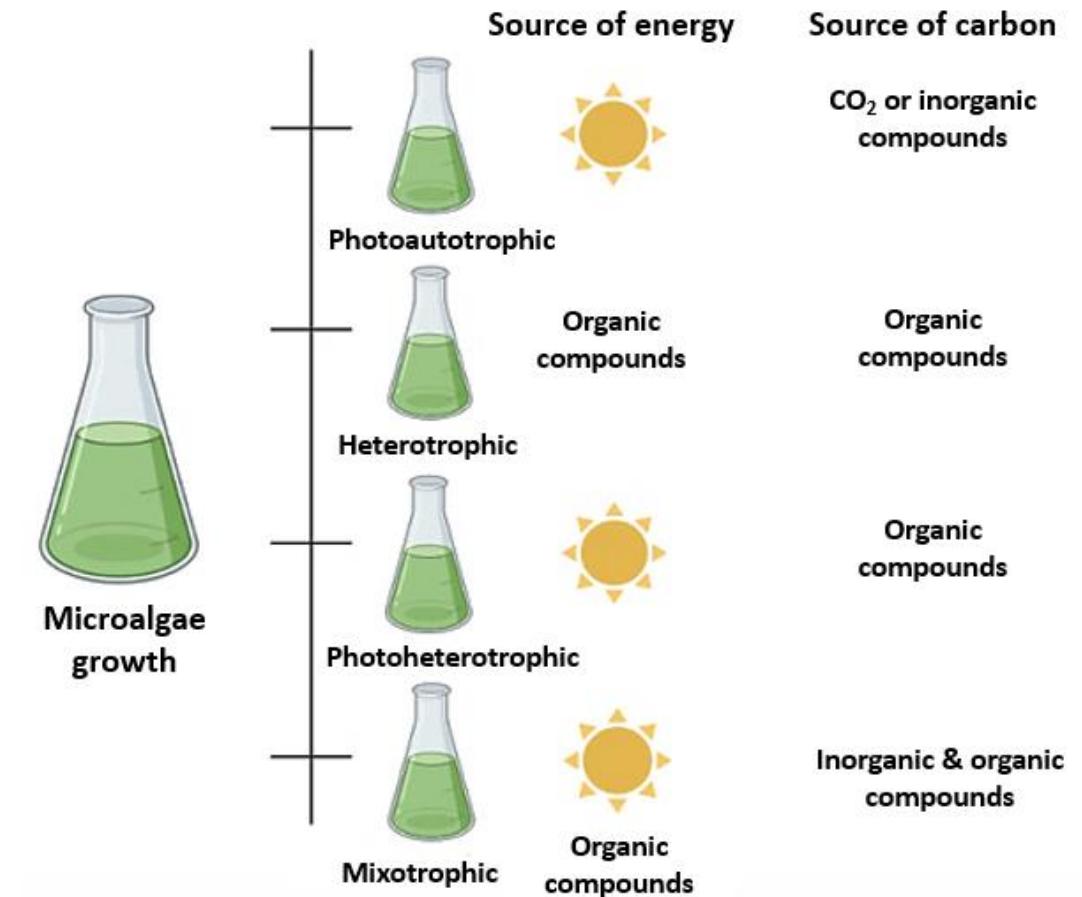


Thermi-Thessaloniki, GR

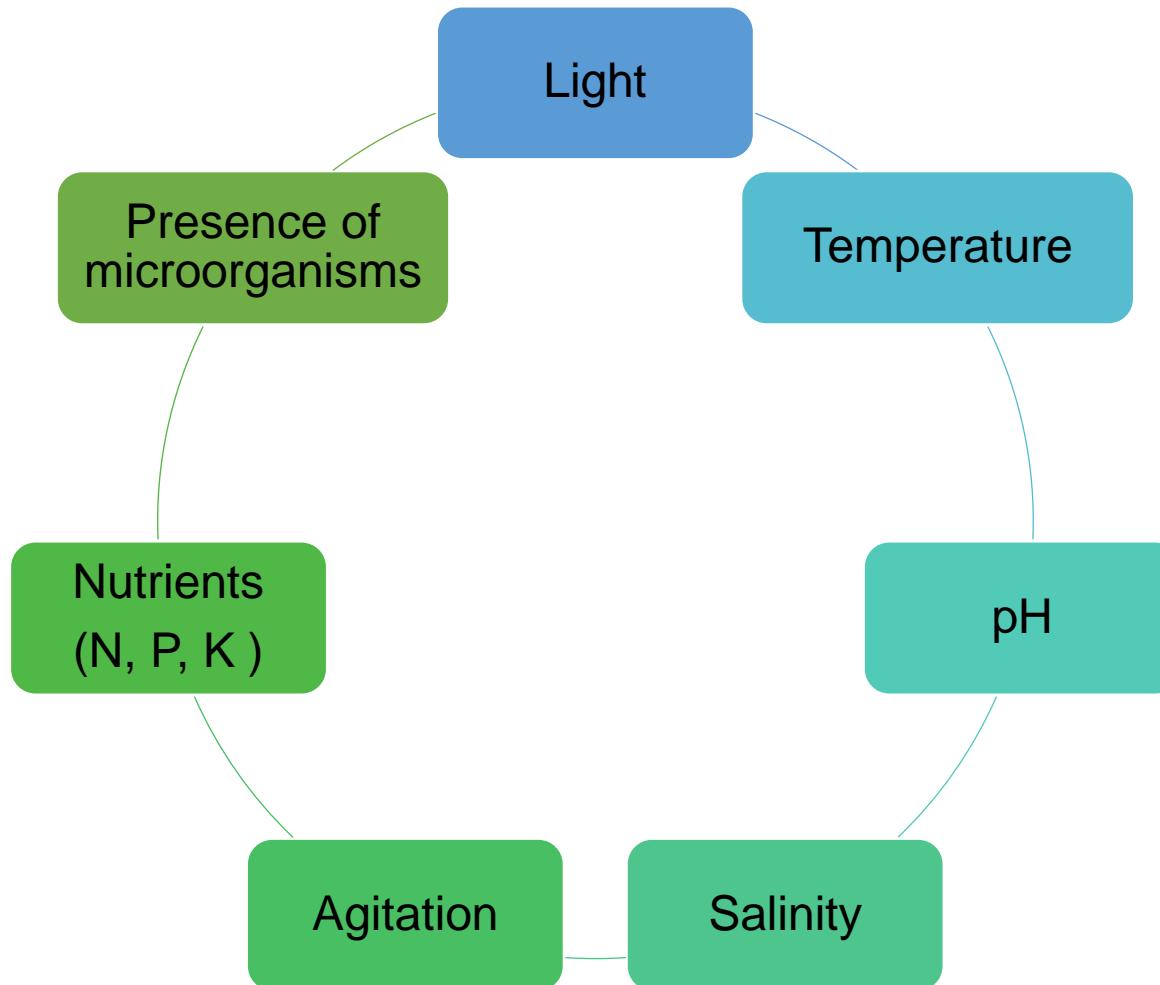
## Microalgae

- Photosynthetic organisms
- Freshwater and marine systems
- No arable land
- Continually processed, not seasonally harvested
- Production various valuable biomolecules

***3<sup>rd</sup> generation biofuel feedstock***

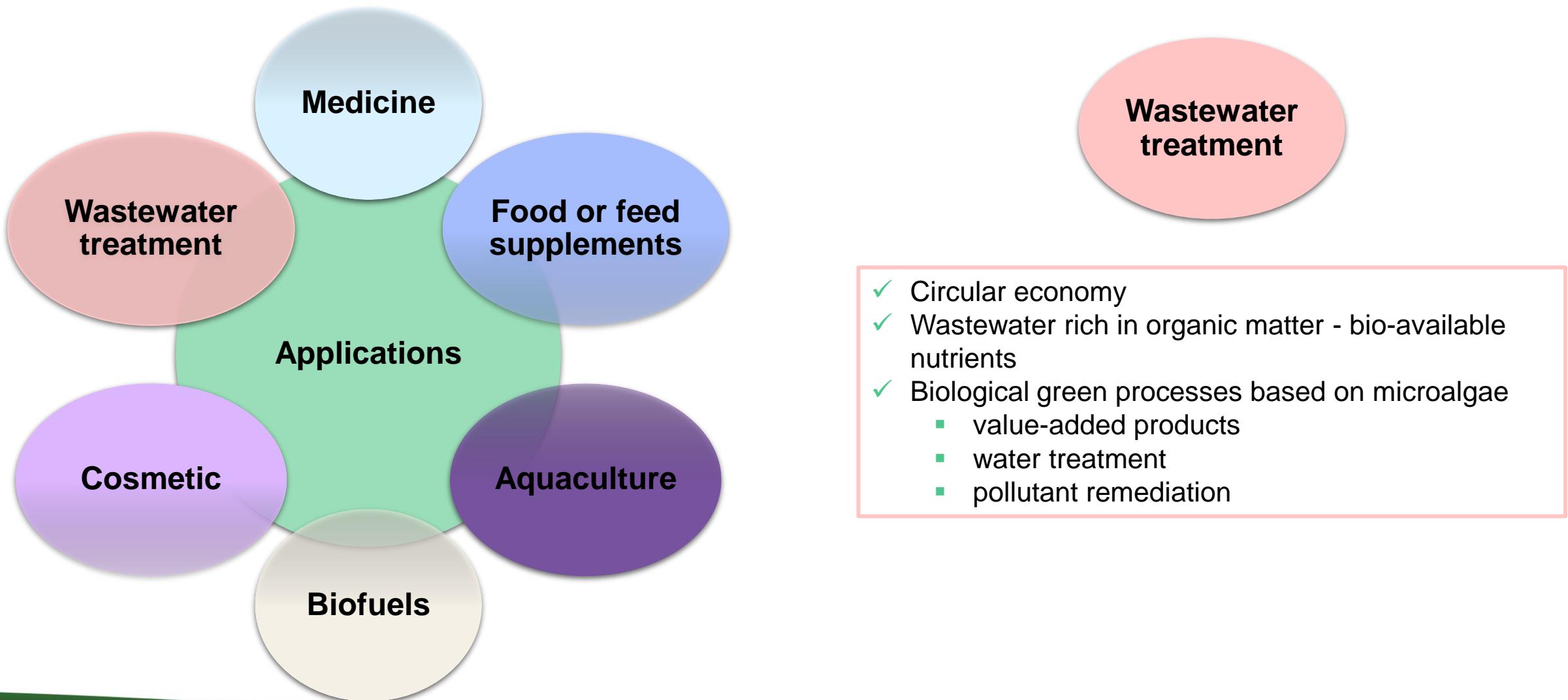


# Cultivation parameters



Parameters	Value
Temperature (°C)	20-30
Light ( $\mu\text{mol}/\text{m}^2/\text{s}$ )	60-350 Cool white light
pH	7-9
Salinity (g/L)	12-40
Agitation (rpm)	100-160
Nutrients	C, N, P, K, ...

# Applications



# Aim & Objectives

**Industrial food wastewater valorization and reuse technique**

**Microtiter microscopic**

**Growth in optimum condition  
(Mixture design)**

**Purification of wastewater**

**High – added value products compounds**



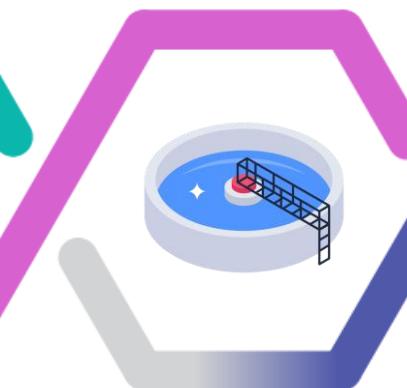
Cultivation of valuable microalgae.



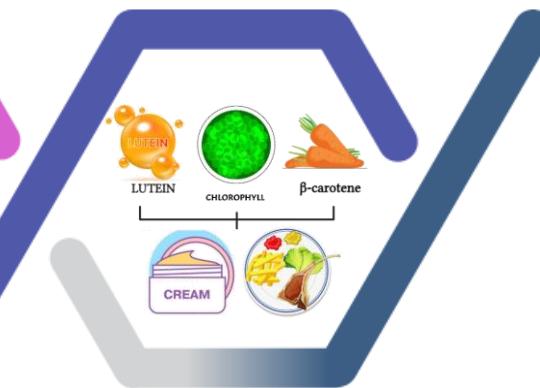
Screening of microalgae strains in microplates



Evaluation of the growth of microalgae under the optimum condition

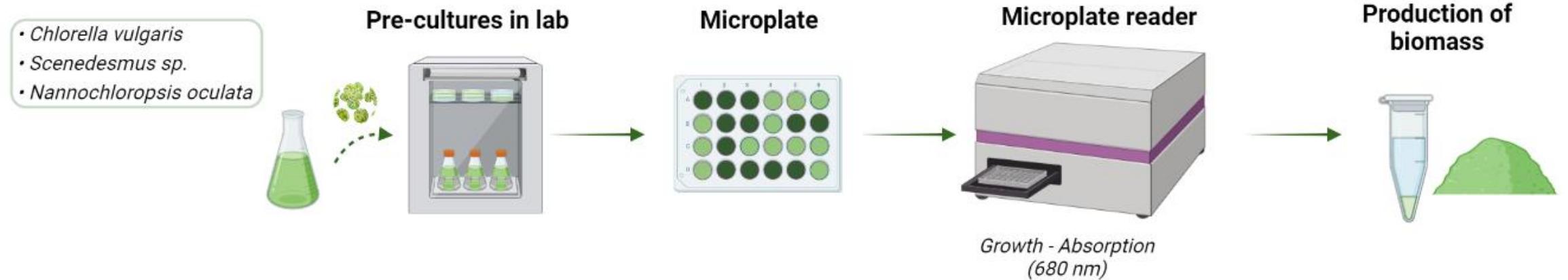


Wastewater treatment

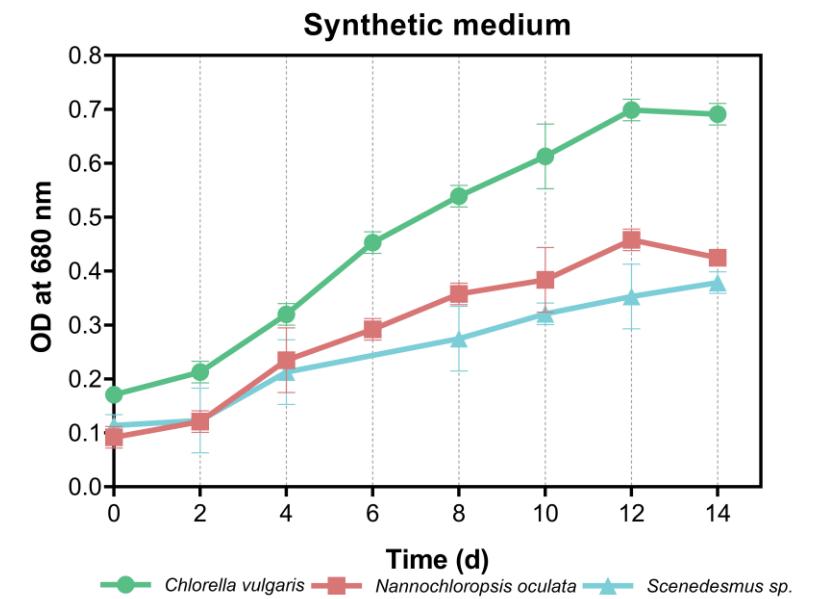


Utilization of biomass in Cosmetics and Food products

# Pre-cultivation of microalgae species



Cultivation conditions	
Metabolism	autotrophic
Media	3N-BBM+V / F2
Temperature	25 °C
Mixing	200 rpm
Light	60-75 µmol/m <sup>2</sup> /s 24h light : dark cycle



# Experimental Design

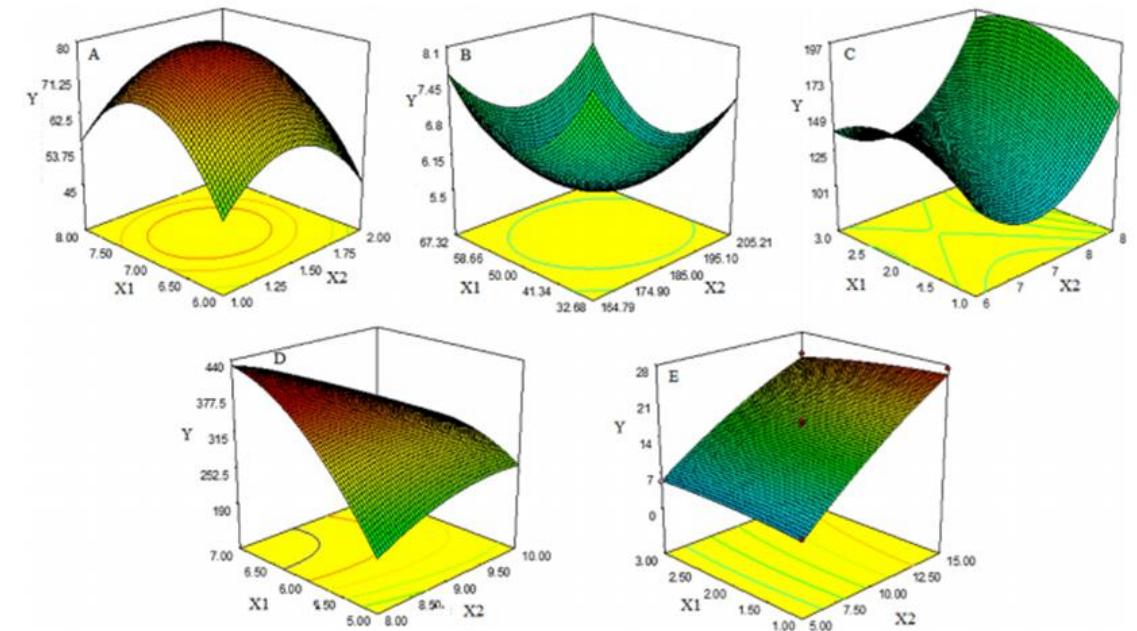
Optimum conditions for growing microalgae in different proportions of liquid waste → greater growth

## Design of Experiments (DoE)



- Objectives**
- Screening
  - Optimization
  - Validation

## Response Surface Methodology (RSM)



# Mixture design for wastewater composition

Factors	Response
Brewery wastewater, ( $X_A$ )	
Expired orange juice, ( $X_B$ )	
Cheese whey, ( $X_C$ )	Optical Density ( $Y_1$ )
Water, ( $X_D$ )	

## Composition range of the four factors

Coded name	Name	Type	Low	High	Units
$X_A$	Brewery wastewater	Mixture	0	100	%
$X_B$	Expired orange juice	Mixture	0	100	%
$X_C$	Cheese whey	Mixture	0	100	%
$X_D$	Water	Mixture	0	50	%

## I-optimal mixture design

Run	$X_A$ (%)	$X_B$ (%)	$X_c$ (%)	$X_D$ (%)
1	50.00	0.00	0.00	50.00
2	1.03	18.74	52.00	28.22
3	25.00	25.00	25.00	25.00
4	23.51	70.80	0.00	5.68
5	35.32	0.00	28.51	36.17
6	2.74	47.36	49.89	0.00
7	31.51	32.36	0.00	36.12
8	25.00	25.00	25.00	25.00
9	0.00	28.19	71.81	0.00
10	20.85	23.53	45.78	9.84
11	0.00	31.63	30.81	37.56
12	28.14	0.00	71.86	0.00
13	0.00	59.82	27.15	13.03
14	17.87	4.00	64.89	13.24
15	0.00	0.00	100.00	0.00
16	77.80	0.00	0.00	22.20
17	66.07	33.93	0.00	0.00
18	17.46	13.29	19.24	50.00
19	0.00	76.87	0.00	23.13
20	35.32	0.00	28.51	36.17
21	0.00	1.71	72.33	25.97
22	62.59	22.52	1.74	13.15
23	8.02	66.67	25.31	0.00
24	20.85	23.53	45.78	9.84
25	3.48	46.41	0.11	50.00
26	43.30	29.41	27.29	0.00
27	31.51	32.36	0.00	36.12
28	100.00	0.00	0.00	0.00
29	67.80	0.00	27.00	5.20
30	25.00	25.00	25.00	25.00
31	4.54	2.84	42.62	50.00
32	0.00	100.00	0.00	0.00
33	62.59	22.52	1.74	13.15
34	43.57	51.95	4.48	0.00
35	43.30	29.41	27.29	0.00
36	43.50	1.38	45.91	9.21

# Experimental procedure for optimum wastewater mixture

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## Screening Experiments

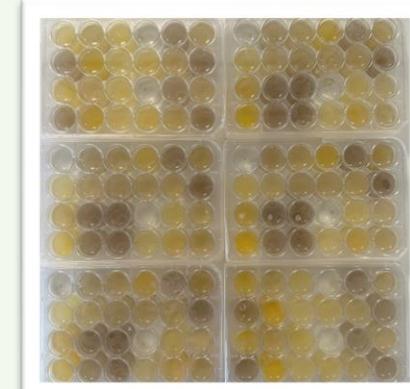
- A total of 36 tests

Run	X <sub>A</sub> (%)	X <sub>B</sub> (%)	X <sub>C</sub> (%)	X <sub>D</sub> (%)
1	50.00	0.00	0.00	50.00
2	1.03	18.74	52.00	28.22
3	25.00	25.00	25.00	25.00
4	23.51	70.80	0.00	5.68
5	35.32	0.00	28.51	36.17
6	2.74	47.36	49.89	0.00
7	31.51	32.00	0.00	36.12
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12	28.14	0.00	71.86	0.00
13	0.00	59.82	27.15	13.03
14	17.87	4.00	64.89	13.24
15	0.00	0.00	100.00	0.00
16	77.80	0.00	0.00	22.20
17	66.00	33.00	0.00	0.00
18	17.46	13.29	19.24	50.00
19	0.00	76.87	0.00	23.13
20	35.32	0.00	28.51	36.17
21	0.00	1.71	72.33	25.97
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29	67.00	0.00	27.00	5.00
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32	0.00	100.00	0.00	0.00
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35	43.30	29.41	27.29	0.00
36	43.50	1.38	45.91	9.21

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## OD tests

- Different wastewater mixture
- Working volume 2 mL
- Cultivation conditions



3

## Microplate Reader

- Daily growth measurement



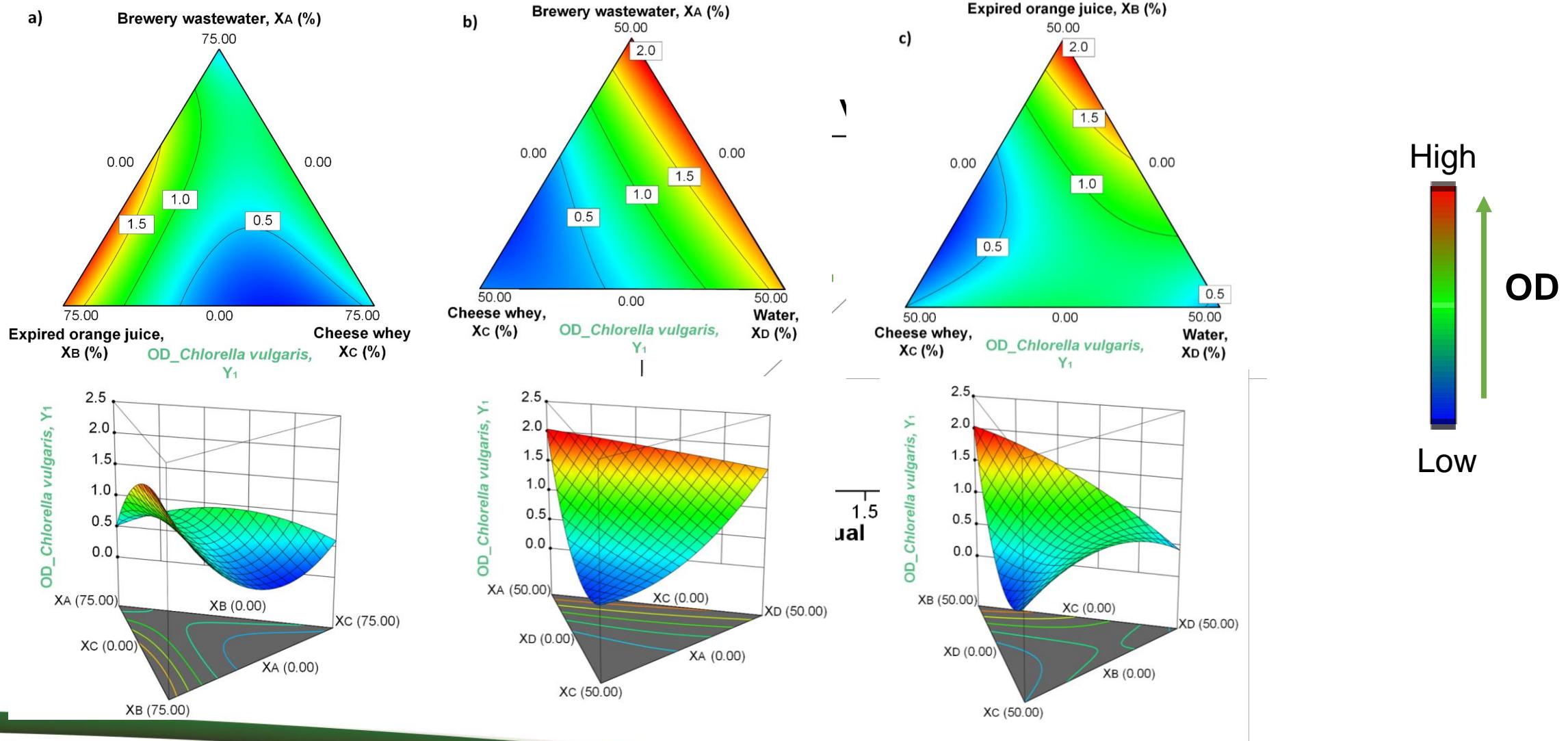
# ANOVA results

Analysis of Variance (ANOVA)  
Statistical significance evaluation  
 $p > 0.05$

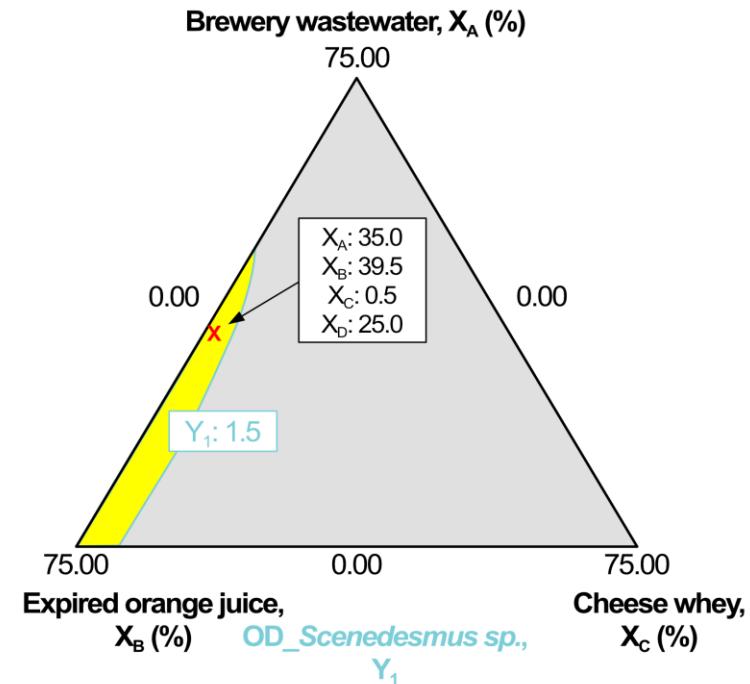
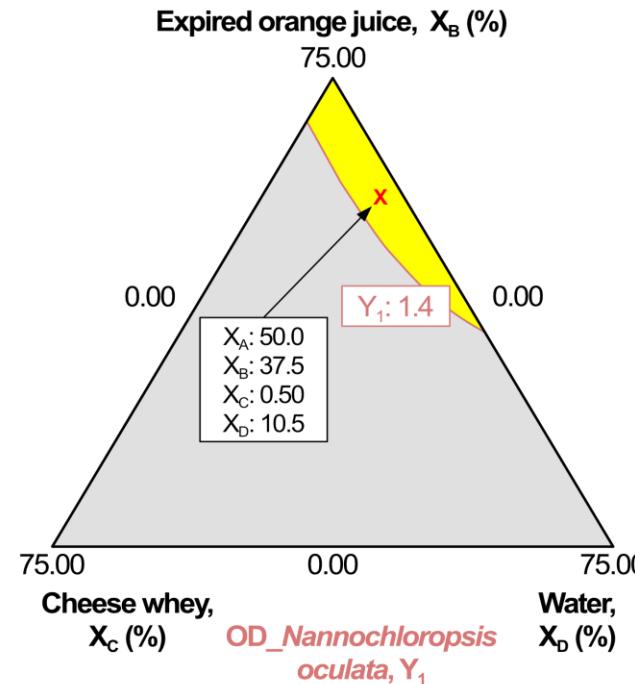
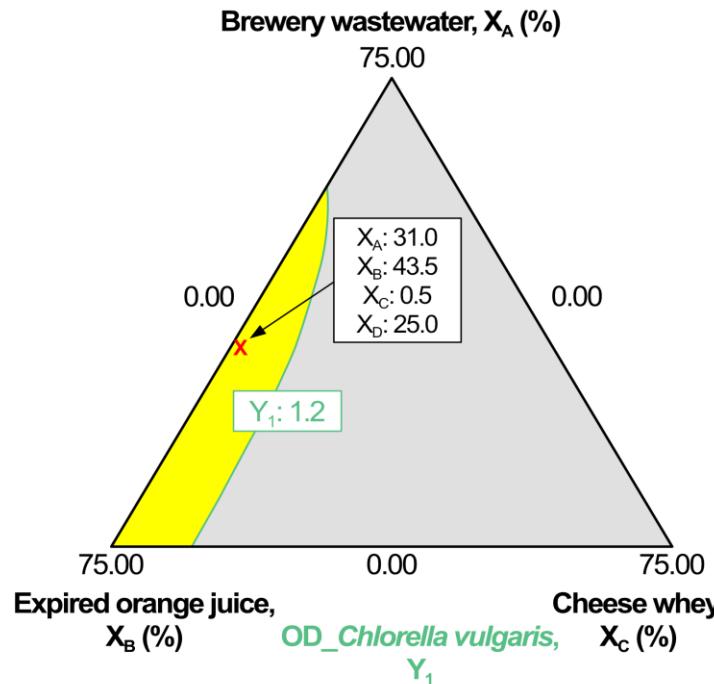
Factors	<i>Chlorella vulgaris</i>	<i>Nannochloropsis oculata</i>	<i>Scenedesmus</i> sp.
	OD at 680 nm ( $Y_1$ )		
R <sup>2</sup>	0.9489	0.9460	0.9222
R <sup>2</sup> <sub>adj</sub>	0.9222	0.9142	0.8911
R <sup>2</sup> <sub>pred</sub>	0.8529	0.7419	0.8113

OD at 680 nm (4 <sup>th</sup> day)			
Run	<i>C. vulgaris</i>	<i>N. oculata</i>	<i>Scenedesmus</i> sp.
1	0.3646	0.2616	0.3016
2	0.3835	0.3611	0.2809
3	0.5411	0.5892	0.5414
4	1.9005	1.6886	1.7499
5	1.1098	0.9329	0.7168
6	0.3072	0.3880	0.2860
7	1.6840	1.5920	1.6080
8	0.5305	0.6492	0.8023
9	0.2197	0.0664	0.0255
10	0.1441	0.0361	0.0861
11	0.3407	0.2982	0.4228
12	0.7733	0.6591	0.6521
13	0.5186	0.5212	0.4721
14	0.4040	0.2606	0.5208
15	0.3291	0.3348	0.4177
16	0.5193	0.3664	0.5607
17	1.8639	1.6302	1.6183
18	0.9016	1.0774	1.0279
19	1.9589	1.6166	1.7044
20	0.9972	0.8204	0.9053
21	0.5914	0.4363	0.5884
22	1.4009	1.4066	1.6314
23	0.7125	0.7556	0.7408
24	0.2928	0.4081	0.4895
25	1.5639	1.7033	1.7038
26	0.2422	0.3012	0.4375
27	1.2340	1.6867	1.5579
28	0.5846	0.2927	0.5528
29	0.5322	0.7417	1.0417
30	0.5398	0.6338	0.6531
31	0.4528	0.4299	0.4840
32	1.5903	1.9810	1.5295
33	1.5265	1.4398	1.5679
34	1.5959	1.4958	1.5668
35	0.3057	0.3085	0.1463
36	0.4601	0.5498	0.5893

# *Chlorella vulgaris* cultivation in food industry wastewater



# Desired Space and selected optimum conditions



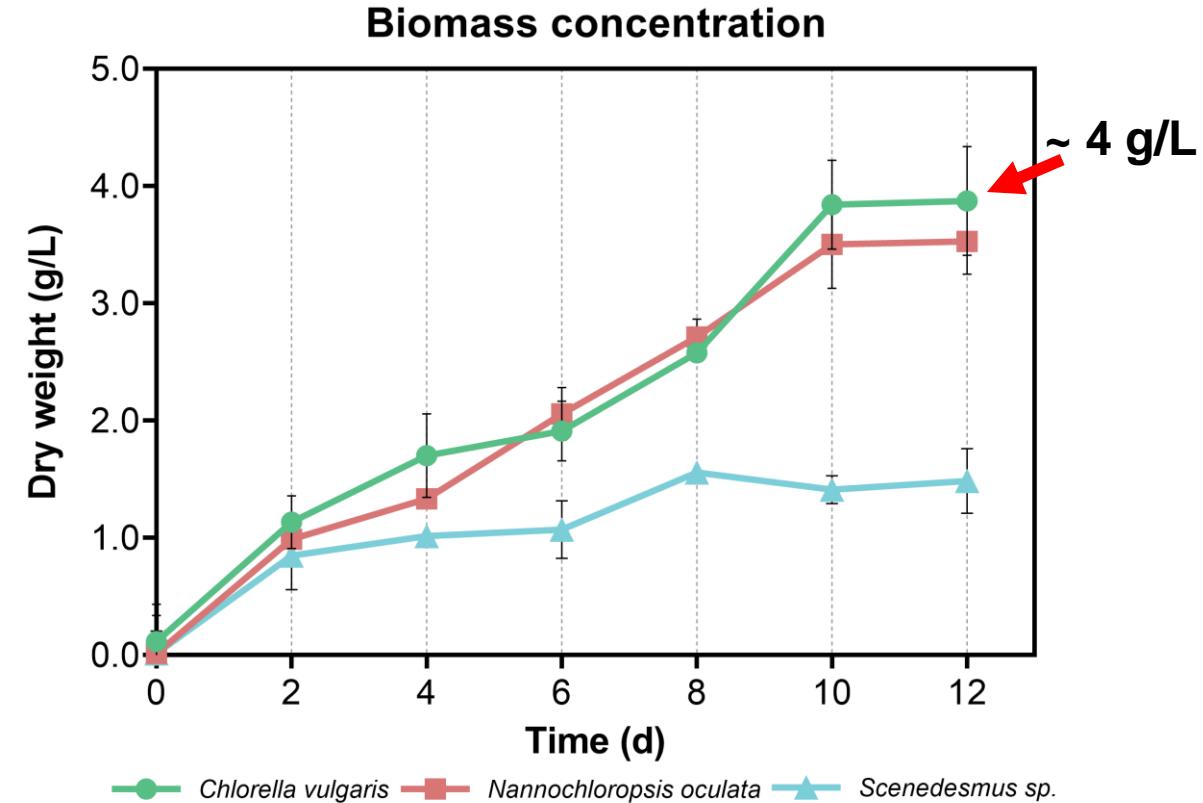
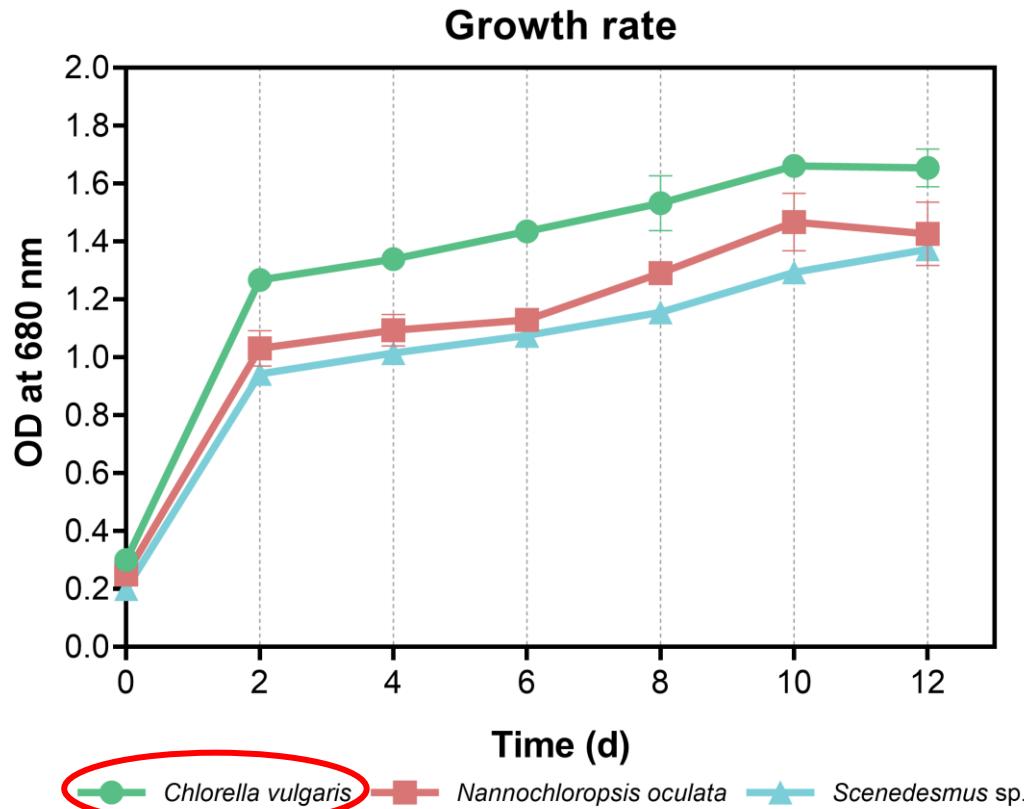
Validation Experiment  
 Between predicted and experimental value

Correlation 95 %

Correlation 83 %

Correlation 97 %

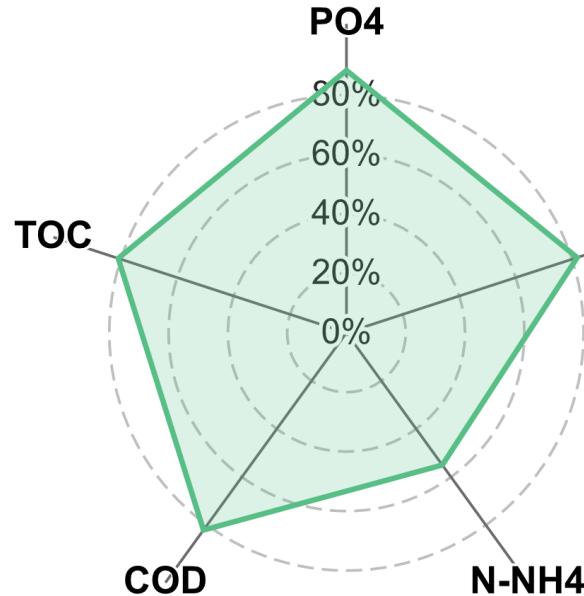
# Microalgae cultivation in optimum WW composition



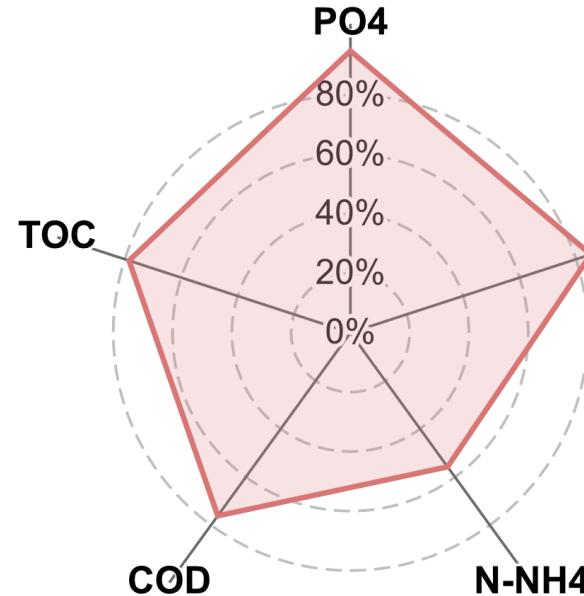
# Food industry wastewater treatment

## Removal of pollutants

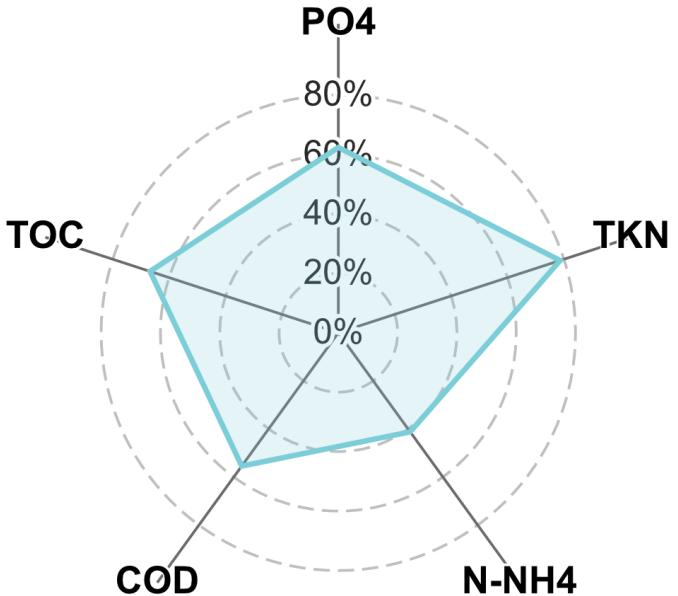
*Chlorella vulgaris*



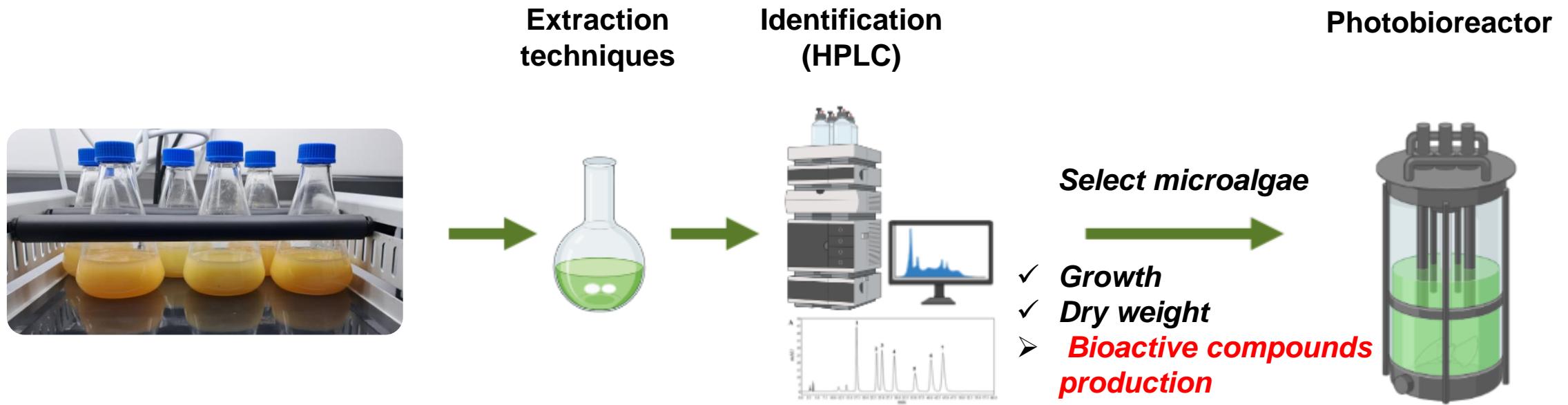
*Nannochloropsis oculata*



*Scenedesmus sp.*



# Ongoing....



## \*Presentation

21 June 2023, 17.30-17.45

I. Papachristou et al. Carotenoid extraction from microalgae cultivated in food industry effluents for the production of cosmeceuticals

# Conclusions

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- ✓ The **model developed was effective** in predicting the response's value with good accuracy.
- ✓ The optimal mixture **mainly contained expired orange juice**, followed by brewery wastewater.
- ✓ Under optimum conditions obtained from the experimental design, the abundance of wastewater nutrients **enhanced the growth of *C. vulgaris*** more than the other two.
- ✓ The cultivation of microalgae had a positive impact on the purification of wastewater. ***C. vulgaris* and *N. oculata* being able to achieve a range of 80-90% removal**, thus enabling a sustainable eco-friendly way for wastewater treatment.

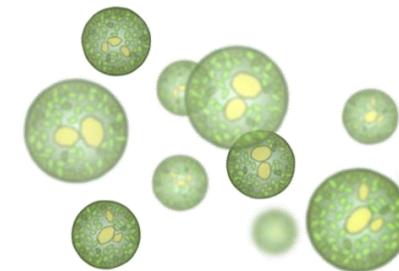
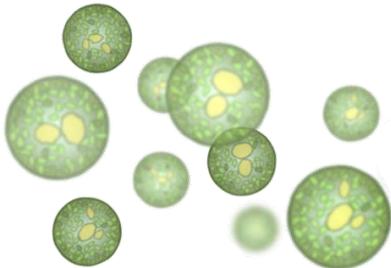
# Acknowledgement



This activity was supported by the project BlueBioChain, Project ID 31, which is co-funded by European Union's Horizon 2020 Grant agreement No. 817992 and national funds General Secretariat for Research and Innovation GSRI, Greece, MIS 5168515/ T12EPA5-00077 under the ERA-Net BlueBio Co-fund.



***Thank you for your attention!!***





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