

10th International Conference on Sustainable Solid Waste Management Chania,  
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# Carotenoid extraction from microalgae cultivated in food industry effluents for the production of cosmeceuticals

I. Papachristou<sup>1</sup>, C. Anagnostopoulou<sup>1,2</sup>, K.N. Kontogiannopoulos<sup>1</sup>, P.G. Kougias<sup>1</sup>

<sup>1</sup> Soil and Water Resources Institute, Hellenic Agricultural Organization Dimitra, Thermi 57001, Greece

<sup>2</sup> Department of Food Science & Technology, Faculty of Agriculture, Aristotle University of Thessaloniki, Greece

 @WMBlab

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 Thermi-Thessaloniki, GR

# Table of Contents

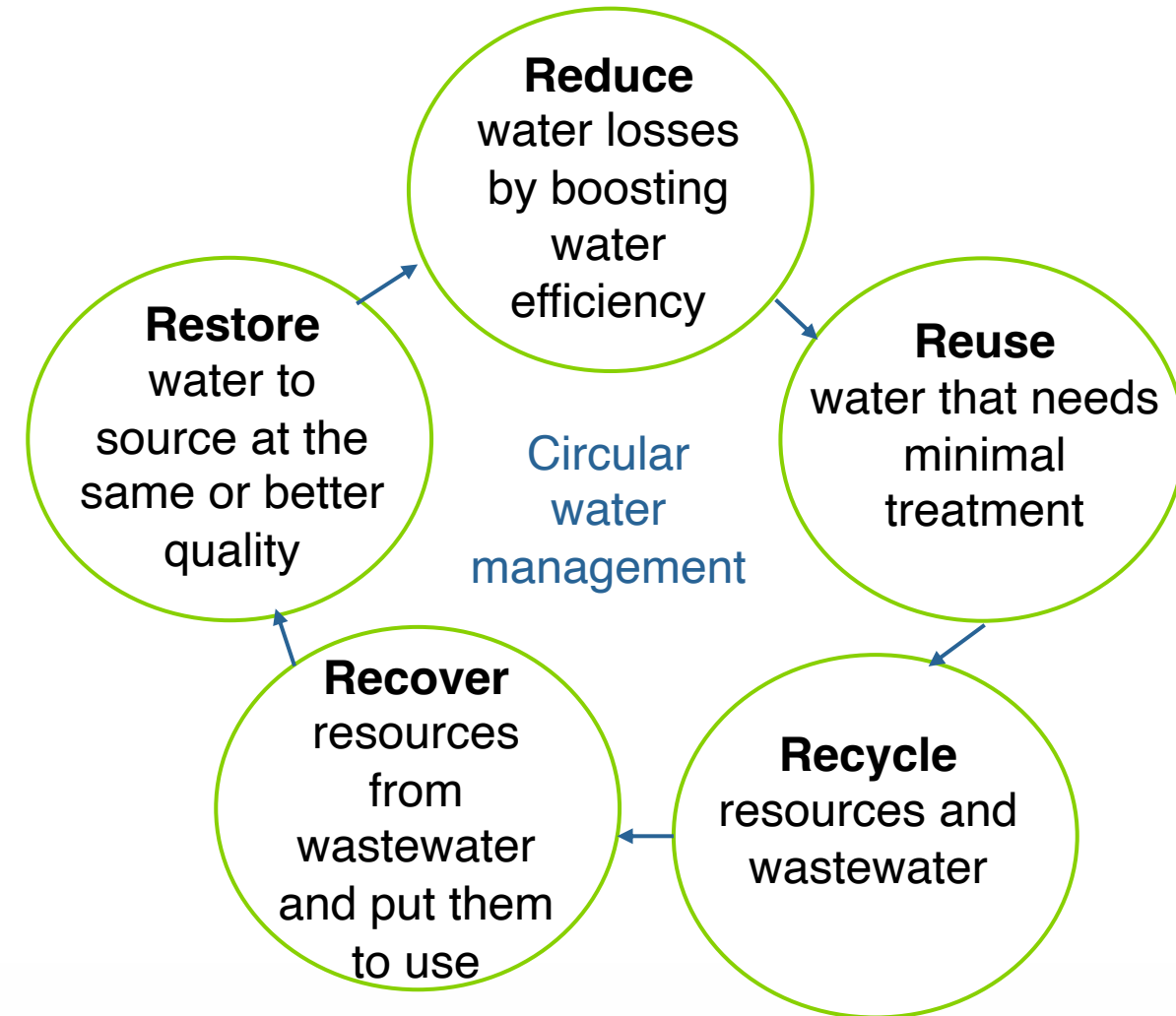
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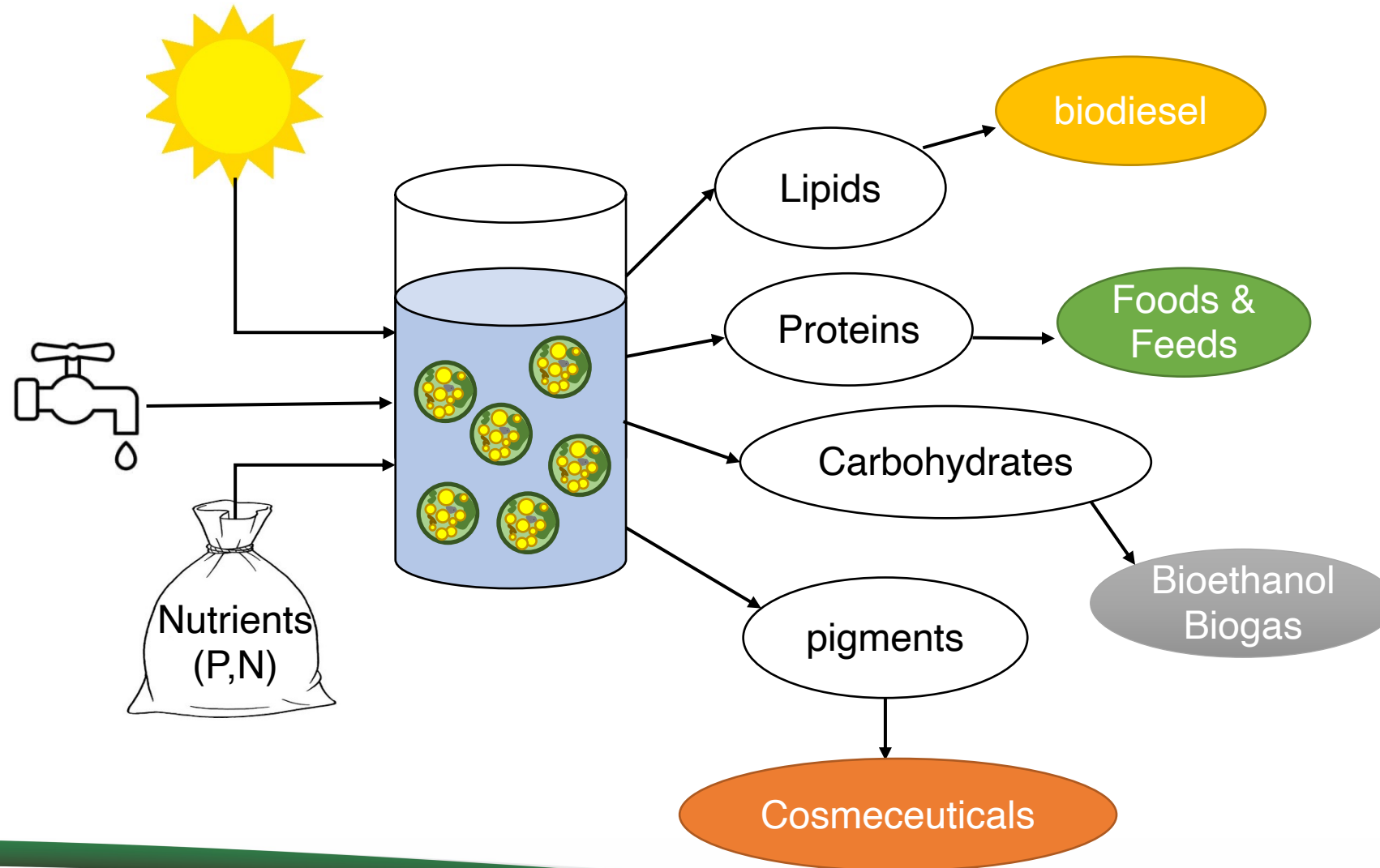
1. Introduction
2. Materials and methods
3. Results
4. Conclusion

# Water and circular economy

- Water is an indispensable resource for any ecosystem.
- Access to clean water is a recognized human right by United Nations
- Up to 30% increase in global water demand by 2050 (Boretti et al, 2019)
- 57% of total water withdrawal in Europe was by Industrial sector (Aquastat)



# Microalgae cultivation

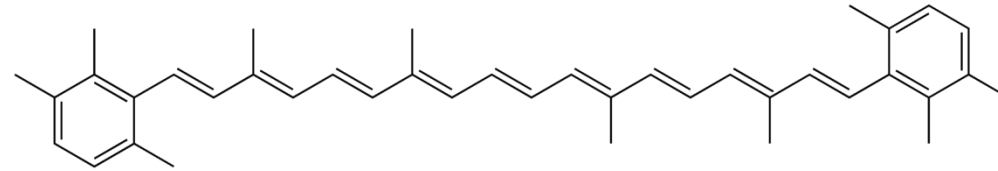


- ✓ Fast growth rates
- ✓ Highly resilient and adaptable
- ✓ Versatile product output

# Carotenoids

## What are they?

- Lipophilic compounds
- Anti-inflammatory and antioxidant activity



## Applications

- Natural food colorants
- Dietary supplements
- Cosmeceuticals

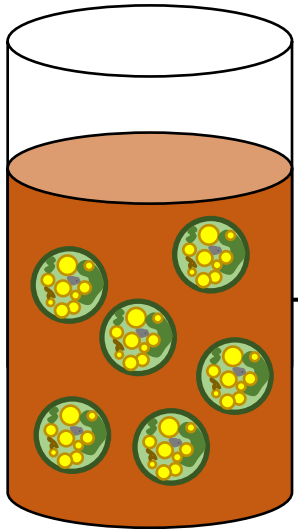


## Extraction

- Organic solvents are typically required (acetone, ethanol, hexane etc)
- Inert conditions

# Aim and objectives

**Wastewater**



**Microalgae**



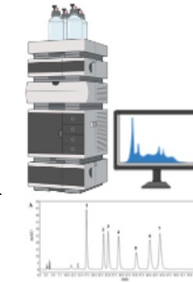
**Extraction methods**

Ultrasound-assisted  
Microwave- assisted  
Direct saponification

**Extract**



**HPLC analysis**



**Cosmeceuticals**



# Materials and methods

- Cultivation medium was a **mixture** of expired orange juice, brewery wastewater and cheese-whey
- Model microalga was *Chlorella vulgaris*
- Pigment extraction took place overnight in inert conditions from lyophilized biomass with chloroform:methanol (2:1, vol/vol)
- Pigment concentration was determined spectrophotometrically according to equations from Dharma et al

$$\text{Chlorophyll } a(\text{Cha}) = 16.72(A_{665.2}) - 9.16(A_{652.4})$$

$$\text{Chlorophyll } b(\text{Chb}) = 34.09(A_{652.4}) - 15.28(A_{665.2})$$

$$\text{Total carotenoid content} = [1000(A_{470}) - 1.63\text{Cha} - 104.96\text{Chb}]/221$$

# Results



# Cultivation in wastewater



**Anagnostopoulou et al**  
 22/06  
 Room 3, Session XI  
 13.15-13.30

1

**Screening Experiments**

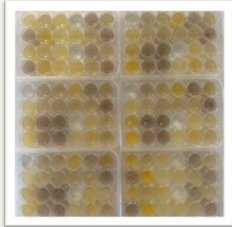
- A total of 36 tests

Run	X <sub>0</sub> (%)	X <sub>1</sub> (%)	X <sub>2</sub> (%)	X <sub>3</sub> (%)
1	50.00	0.00	0.00	50.00
2	11.00	10.74	0.00	78.26
3	25.00	25.00	0.00	50.00
4	23.51	73.30	0.00	3.19
5	25.32	0.00	28.91	45.77
6	17.74	47.38	0.00	34.88
7	31.51	32.38	0.00	36.12
8	20.00	20.00	20.00	40.00
9	0.00	28.19	71.81	0.00
10	20.00	21.53	40.78	17.69
11	0.00	31.03	30.81	37.96
12	28.14	0.00	71.86	0.00
13	0.00	39.52	27.15	33.33
14	17.07	4.00	64.93	14.24
15	0.00	0.00	100.00	0.00
16	17.80	0.00	0.00	82.20
17	69.07	33.55	0.00	0.00
18	17.45	13.23	0.00	69.32
19	0.00	78.87	0.00	21.13
20	30.00	1.00	0.00	68.99
21	0.00	0.00	73.25	26.75
22	62.50	22.53	1.74	13.15
23	0.00	69.07	31.93	0.00
24	20.38	21.53	40.78	17.31
25	1.42	48.11	51.11	30.00
26	43.30	28.41	27.29	0.00
27	31.51	32.38	0.00	36.11
28	100.00	0.00	0.00	0.00
29	07.80	0.00	07.80	83.40
30	25.00	25.00	25.00	25.00
31	14.04	7.84	42.92	35.20
32	0.00	100.00	0.00	0.00
33	20.00	20.00	17.4	42.59
34	43.87	51.95	4.48	0.00
35	43.30	28.41	27.29	0.00
36	43.50	1.38	45.91	9.21

2

**OD tests**

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



3

**Microplate Reader**

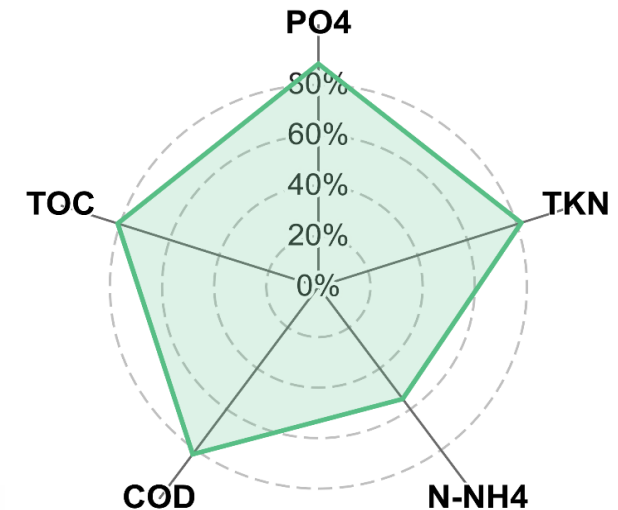
- Daily growth measurement



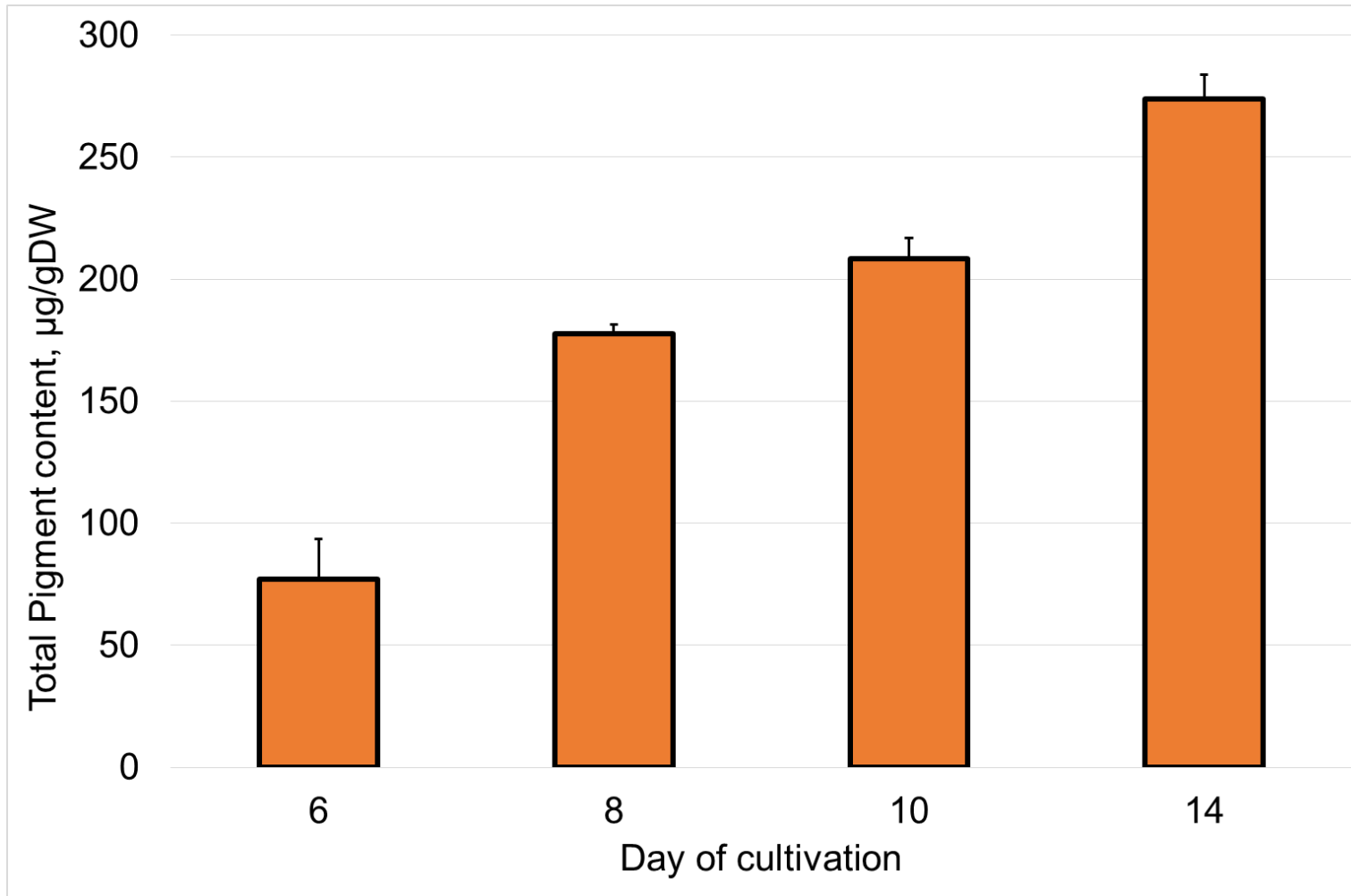

## Cultivation medium

Wastewater type	Composition (%)
Expired orange juice	43.5
Brewery wastewater	31.0
Cheese whey	0.5
Water	25.0

## phycoremediation

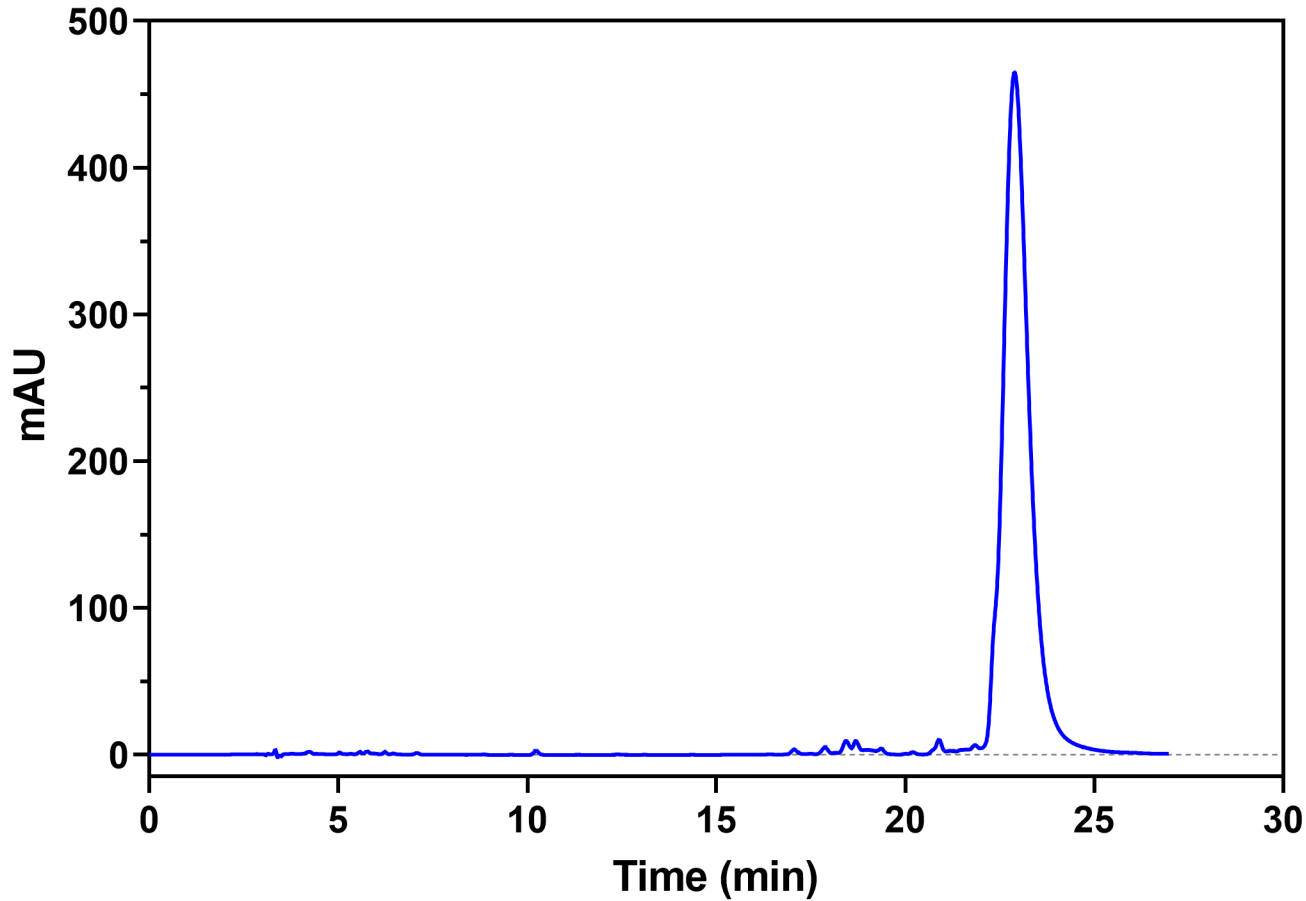


# Pigment extraction



- ✓ Spectrophotometric results from lyophilized biomass
- ✓ Pigment content was steadily increasing
- ✓ At the end of cultivation, pigment content was 270  $\mu\text{g/g}_{\text{DW}}$
- ✓ Preliminary results on **wet** biomass with ethanol:hexane indicate a ~65% extraction efficiency.

# HPLC profile



- ✓ Ongoing work
- ✓ More powerful tool for identification and quantification of pigments
- ✓ So far, 4 individual pigments have been identified: chlorophyll a, chlorophyll b, lutein and  $\beta$ -carotene

# Conclusions

- *C. vulgaris* was cultivated in a mixture of expired orange juice, brewery wastewater and cheese-whey
- Phycoremediation of food industry effluents was proven feasible with microalgae
- Pigment content was 270  $\mu\text{g/g}_{\text{DW}}$  after 14 days of cultivation
- Preliminary HPLC results indicate the presence of chlorophyll a, chlorophyll b, lutein and  $\beta$ -carotene.

# Future steps

- Optimization of carotenoid extraction (pretreatment method, solvents etc)
- Determination of the biological activity (total phenolic content, antioxidant activity etc)
- Skin cream production

# Acknowledgement

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Thank you for your attention!

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