Corso di Dottorato di Ricerca in Scienze della Vita e dell'Ambiente - Ciclo XL

Establishment of a circular supply chain through the cultivation of algae using potential by-products from the agri-food industry

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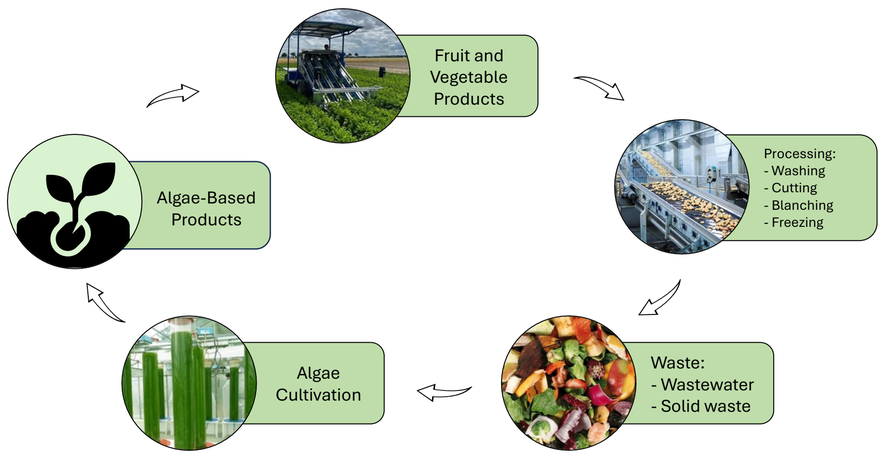
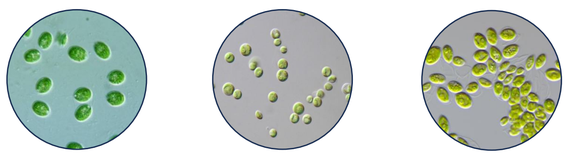
Aim of the project

The goal of this project is to **valorize agri-food industry by-products** to foster sustainability and drive the transition towards a circular economy model. Within this context, **microalgae** represent a promising **biotechnological platform** for the **production of high-value compounds**. Agri-food wastewaters and residues can be exploited as an alternative culture medium, offering a sustainable source of water and essential nutrients for microalgal cultivation.



Species

For this study, three freshwater **Chlorophyta** were selected. Their ability to perform **mixotrophic metabolism** makes them particularly suitable for **wastewater cultivation**, due to their high resilience and rapid growth under variable nutrient conditions.

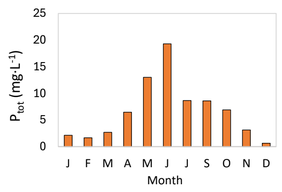


Chlamydomonas reinhardtii | *Chlorella vulgaris* | *Tetradesmus obliquus*

Culture medium selection

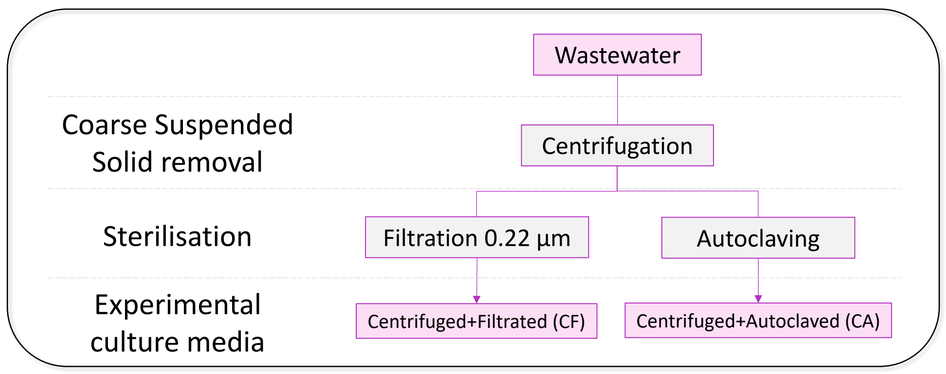
Major bottlenecks in microalgae cultivation in wastewater:

- Seasonal variability in wastewater composition → Variable nutritional profile
- Biological contamination → Grazing, competition for nutrients
- High level of suspended solids → Low light penetration

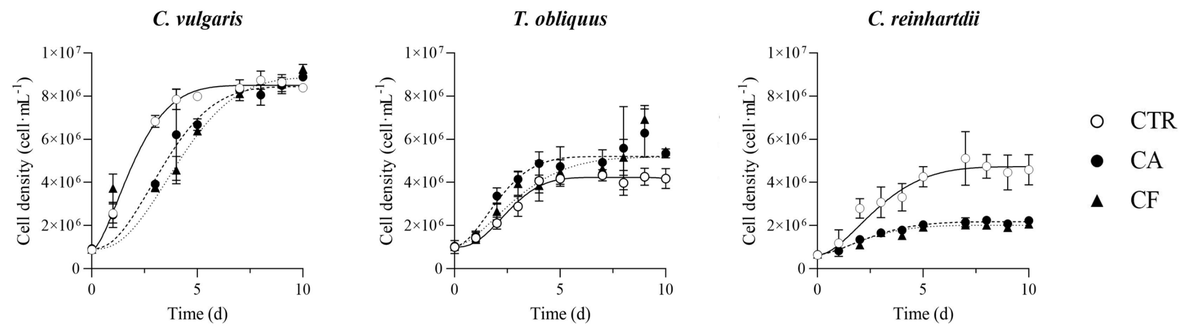


The first wastewater sampling was conducted in **December** 2024, when **nutrient concentrations** typically reach their annual **minimum**

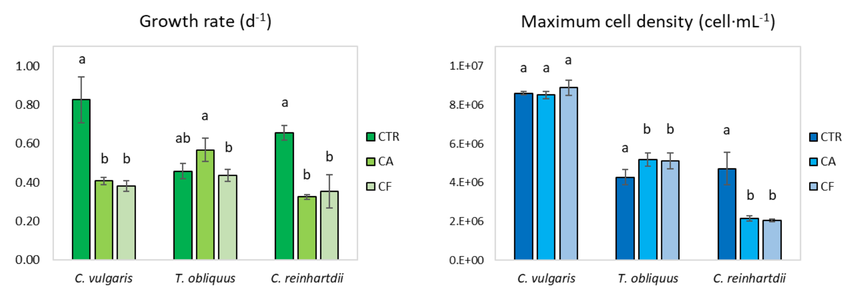
The initial step was to determine which **pretreatment method** should be applied to the wastewater **to enable microalgal growth**



The **effect of the different pretreatments** on wastewater was evaluated on the growth of **non-acclimated cells**



Slight differences were observed for ***C. vulgaris*** and ***T. obliquus*** between the two experimental media and the control. By contrast, ***C. reinhardtii*** exhibited markedly lower growth rate and maximum density compared to those of the control.



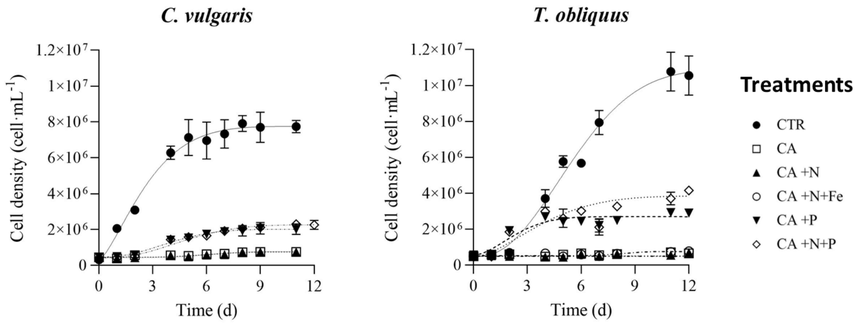
Growth did not differ significantly between the two pretreatment methods

CA medium was **selected** for the next phase, as autoclaving provided greater protection against contamination under laboratory conditions

Biomass quality in response to nutrient limitation

During the acclimation phase in the selected experimental medium (CA), ***C. vulgaris*** and ***T. obliquus*** survived for three generations before ceasing growth, whereas ***C. reinhardtii*** did not survive

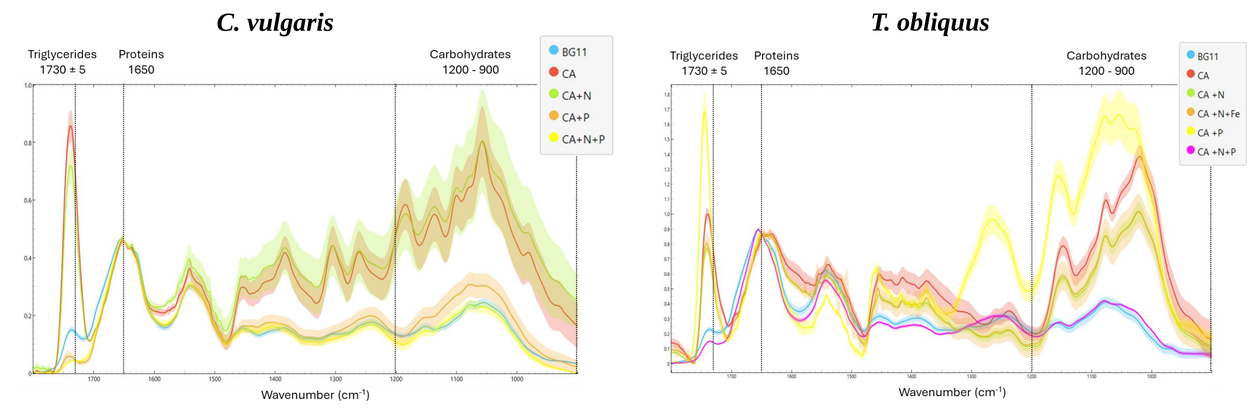
CA medium was supplemented with **Nitrogen (N)**, **Phosphorus (P)**, and **both nutrients** to test whether they were limiting algal growth



Cultures supplemented with **P** and **both N and P**, demonstrated **improved growth rate** and **maximum density**. The addiction of **Iron** (**Fe**), for improving nitrogen assimilation, showed no beneficial effect.

Preliminary results indicate that algal cells experienced **nutrient limitation**, particularly by **phosphorus**.

The **macromolecular composition** of whole cells was characterized using **FTIR spectroscopy**. Relative abundances of the main macromolecular pools in the biomass were compared by normalizing to proteins



Biomass cultivated in **CA medium** without nutrient supplementation, or supplemented with **N** and **N+Fe**, exhibited higher **lipid/protein** and **carbohydrate/protein ratios** than the other treatments.

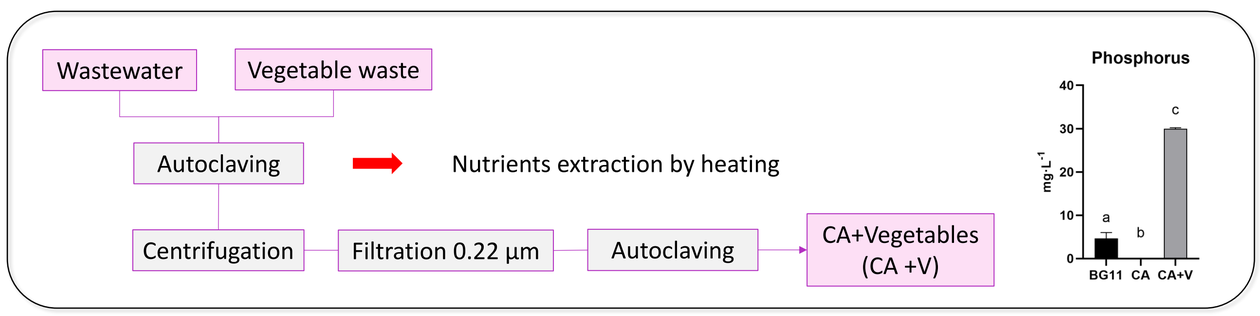
When adding **P**, biomass quality was comparable to that in control condition (BG11).

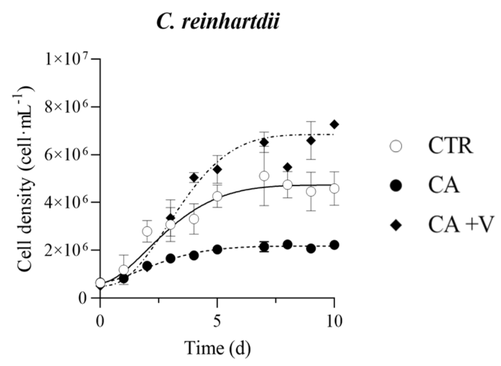
Cells experiencing **nutritional stress** activate the synthesis of **reserve compounds**

Nutrient supplementation strategy

To address nutrient deficiency, the **supplementation of CA medium with nutrients extracted from solid vegetable residues** is currently being explored.

This strategy aims to ensure sustained, year-round productivity.





C. reinhardtii exposed to **CA medium enriched with vegetable residues (CA +V)** grew better than in the other conditions