Evaluation of light quality, temperature and nutritive deprivation impact onto starch accumulation in *Chlorella vulgaris*

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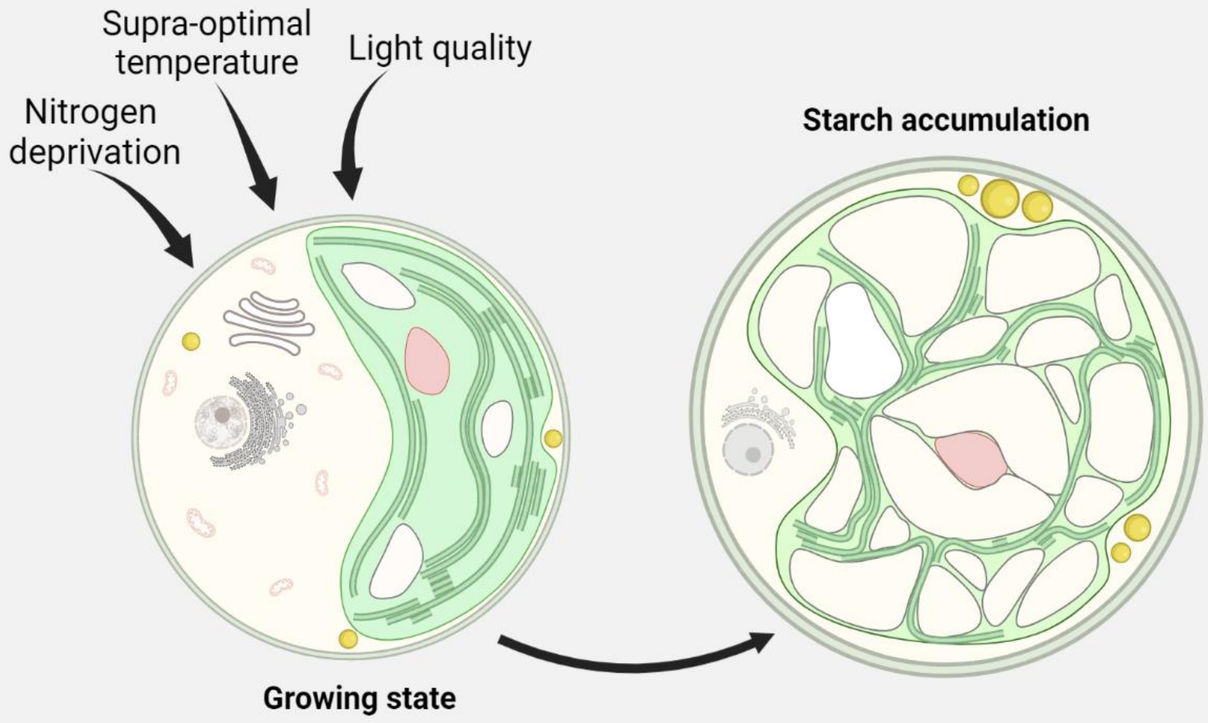
Background

- Microalgae are an appropriate source of starch for bioplastic production, as explored in the Nenu2PHAr and Sealive projects.
- Green microalgae can drastically increase their starch productivity under specific abiotic factors[1]. Even though nutrient deprivation is classically used at high-scale, supra-optimal temperature[2,3] and light quality[4] are alternative ways of inducing starch accumulation.

Aims

- Evaluate the relevance of supra-optimal temperature and light-quality onto starch accumulation in the industrial relevant strain *Chlorella vulgaris* CCALA924[1].
- Compare alternative starch-inducers with classic nutrient deprivation.

Graphical abstract



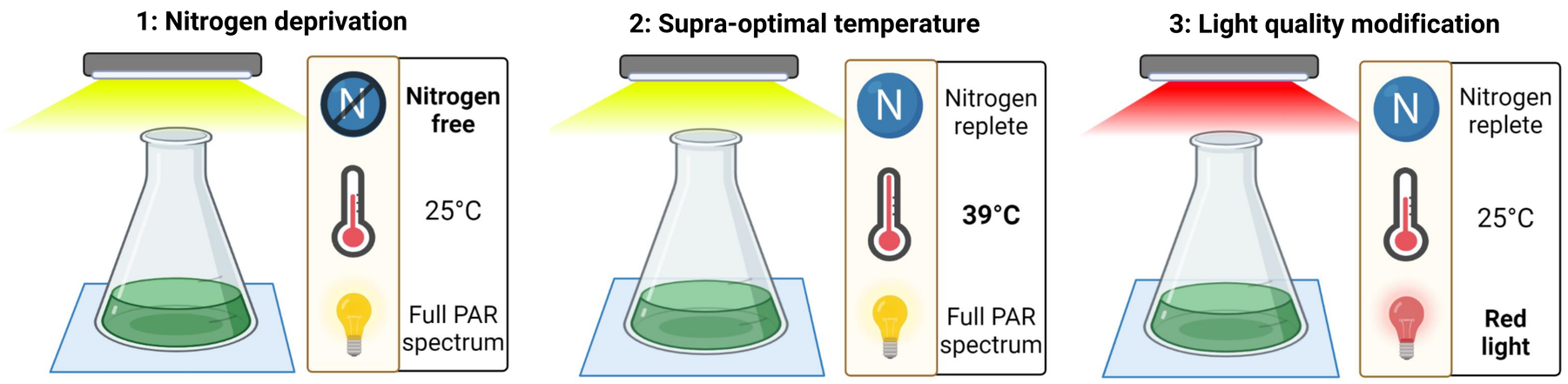
Material & Method

Culture

- *Chlorella vulgaris* CCALA924
- Initial concentration 1g/L
- Beijerinck Media adapted with $NaNO_3$
- 200|0µE/m²/s for 11|13h

Monitoring

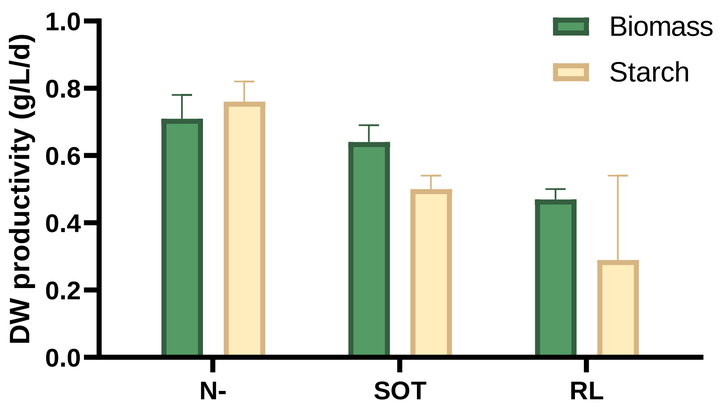
- Dry weight of rinsed biomass at start and end of cultures
- Batch duration : 3 days for nitrogen deprivation and supra-optimal temperature, 4 days for red light



Results

Nitrogen depletion induced the best starch productivity

A.



B.

	%Starch equivalent* in biomass (DW)
Nitrogen depleted	73.8±5.1
SOT	50.2±0.6
RL	39.2±1.4

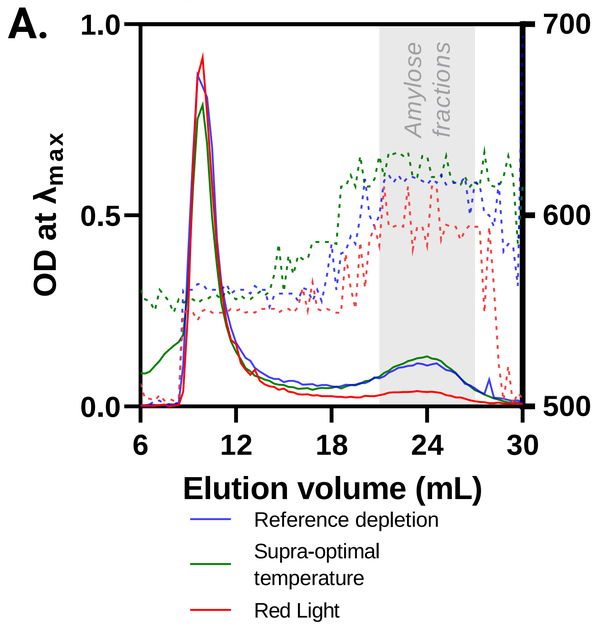
*Starch equivalent = Total carbohydrates x 0,9

Total carbohydrates were quantified with acid sulphuric-phenol colorimetric method using a glucose standard.

Fig. 1: (A) Comparison of biomass and starch productivity. (B) Final starch content in the biomass. N-: Nitrogen depletion, SOT: Supra-optimal temperature, RL: Red light

Light-quality modification led to low amylose content

A.



B.

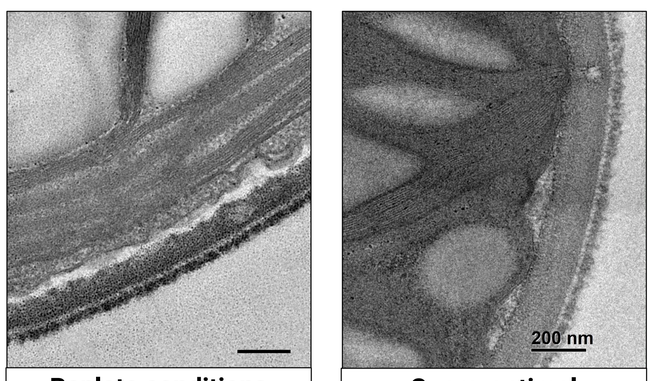
	%Amylose in starch (DW)
Reference depletion*	11.5%
S.-O. Temperature	13.8%
Red Light	Below detection limit

*Reference depletion is a reference data of nitrogen depletion from another experiment

Fig. 2: (A) Separation of amylopectin and amylose by size exclusion chromatography (full lign: OD, dashed ligh: λ_{max}, grey area: amylose) and (B) Amylose quantification.

Supra-optimal temperature conditions thickened cell walls

A.



B.

	Cell wall thickness (nm)
Control N+	78±8nm
S.-O. Temperature	146±7nm

Mean of 25 measurements done on 5 cells with ImageJ.

When cultivated outdoors, nitrogen depleted cells had a cell wall thickness of 110±20nm.

Fig. 3: (A) TEM observations of *Chlorella vulgaris* cells after exposure to supra-optimal Temperature and control conditions. (B) Measurement of cell wall thickness.

Conclusion

- Supra-optimal temperature and light-quality modification were attested to trigger high level of starch accumulation in *Chlorella vulgaris*.
- Interestingly, red light induced very low amylose content in starch despite a relatively high accumulation. Most likely, light-quality modification trigger different pathways that could be further investigated.
- The type of starch-inducer probably has an effect on cell wall thickness, which could hinder cell breakage for starch recovery.

1 Bránykóvá I, et al. Biotechnol Bioeng. 2011;108(4):766-76.
2 Zachleder V, et al. Cells. 2021;10(7):1806.
3 Ivanov IN, et al. Cells. 2021;10(5):1084.
4 Yuan Y, et al. Video-poster. IBEC Congress 2021.

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