CYANOBACTERIA PLATFORM OPTIMISED FOR BIOPRODUCTION – CYAO

Pilot scale cultivation of engineered cyanobacteria for astaxanthin production

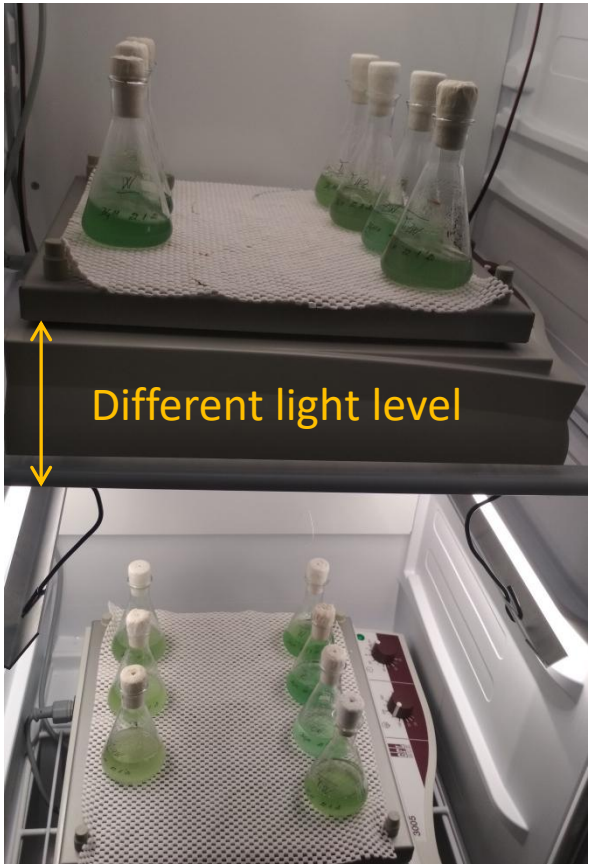
With help of Ing. Jiří Kopecký Ph.D.
and Elizabeth Figueroa M.Sc.

Starting cultures

- *We received 6 strains from which 3 were temperature inducible, thus called „Ti“.*
- *When grown in a stock culture. Ti strains were cultivated in 18 °C room and those without this mutation in 28 °C cultivation room.*
- *Also grown on plates with antibiotics.*

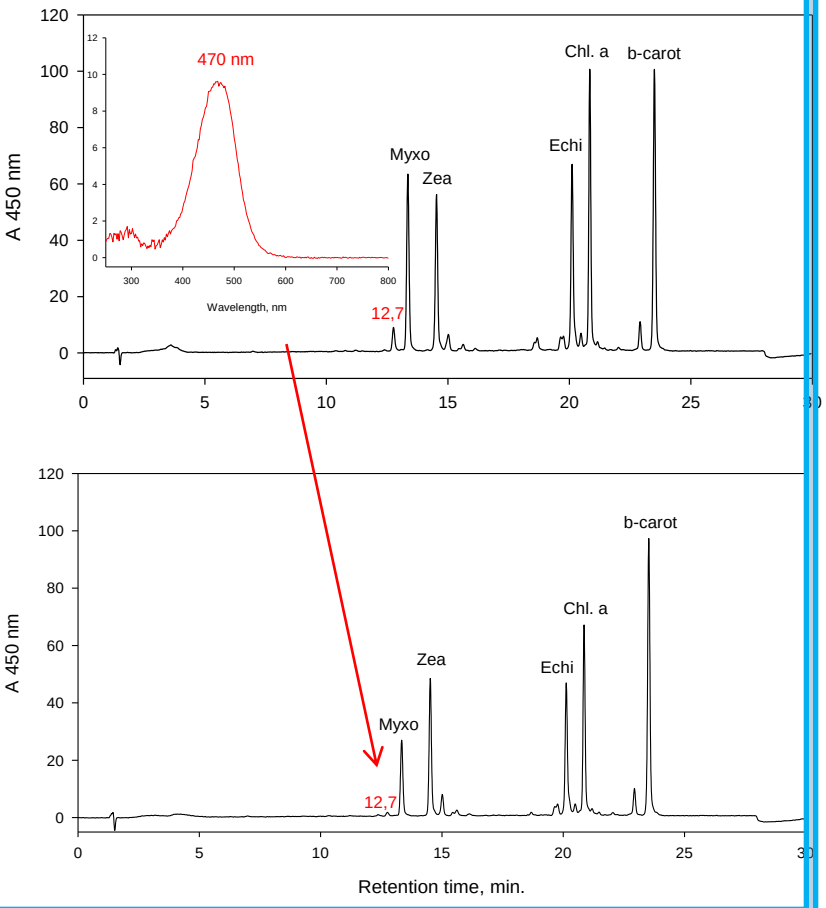
Choosing the most promissing strain.

- *For the experiment the strains were inoculated from plate and after 3 dilutions they were placed into the incubator with temperature set to 36 °C.*
- *All the light conditions were continuous LED panels.*
- *Tested:*
  - *Different light intensities.*
  - *Different start of the culture.*

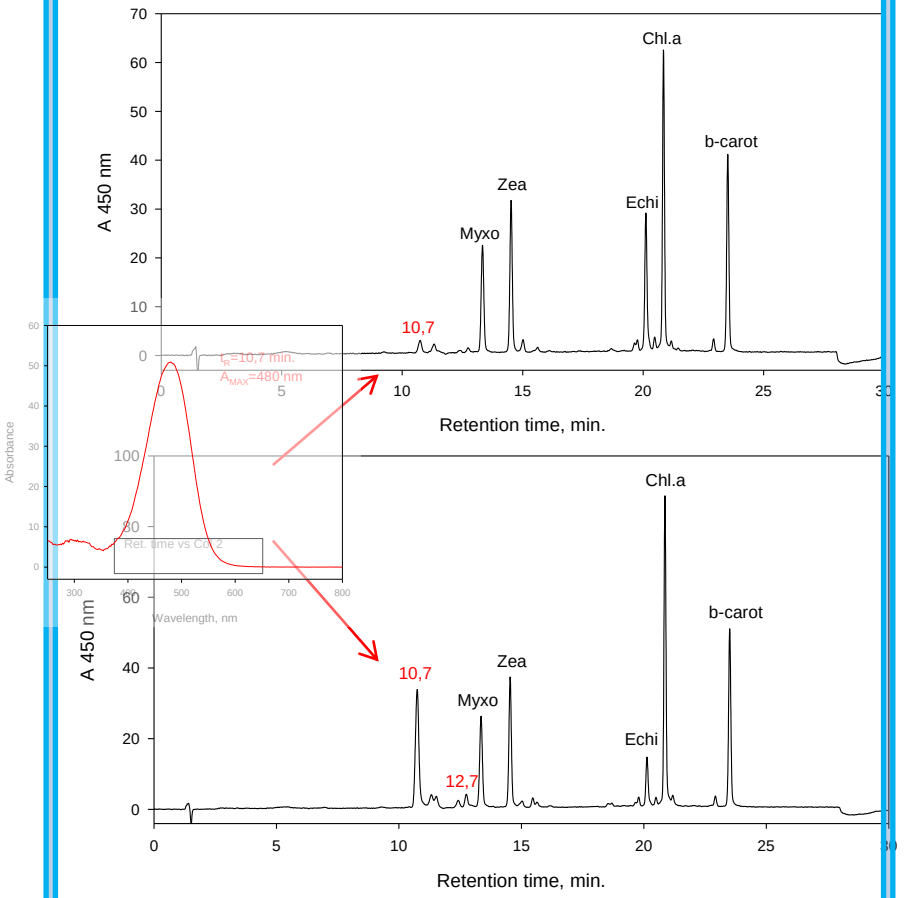


Comparison of the Astaxanthin production in testing cultivation

Z and ZW – 12.7 retention time



TiZW and TiWZ – 10.7 ret. time



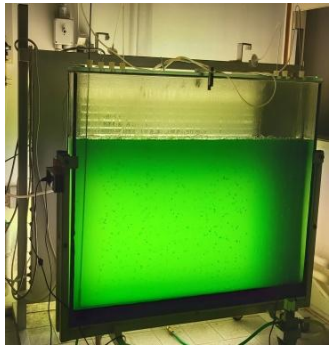
TiWZ vs. TiZW and 50 vs. 100 µE

50 µE/ Strain	D.W. [$g.L^{-1}$]	Ret. Time [min.]	Compound	Peak area [480 nm]	$\mu g.ml^{-1}$	%
TiZW	0.31	10.5	Asta	4.3	0.017	0.0014
TiWZ	0.64	10.5	Asta	13.3	0.054	0.0021

- From this comparison TiWZ was chosen as the optimal candidate for Astaxanthin production, under 100 µE illumination.

100 µE/ Strain	D.W. [$g.L^{-1}$]	Ret. Time [min.]	Compound	Peak area [480 nm]	$\mu g.ml^{-1}$	%
TiZW	0.65	10.5	Asta	484.9	1.971	0.076
TiWZ	0.63	10.5	Asta	875.3	3.558	0.14

Upscaling of TiWZ



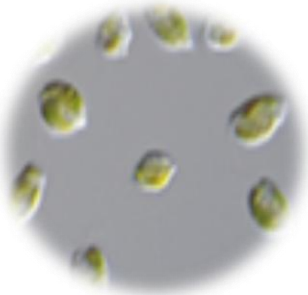
The culture was grown under standart conditions to reach volume like 5 L with optical density 0.3 and then transferred into bigger cultivator. At the start the culture had dry weight 0.13 g/L.

- It seemed to be easy. but:
  - Golden algae (*Ochromonas danica)*
  - protozoa (*Colpoda steinii*)

Ochromonas danica :

80 L

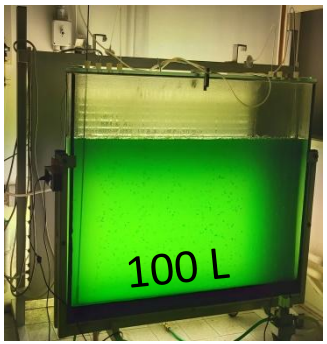
+



- Why?
  - The golden algae consume smaller organisms with exponenciall likeliness. The smaller the more probably will be eaten.

Protozoa contamination

protozoa: the ciliate
Colpoda steinii



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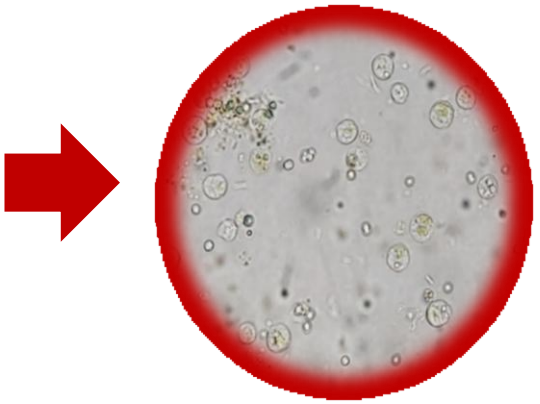
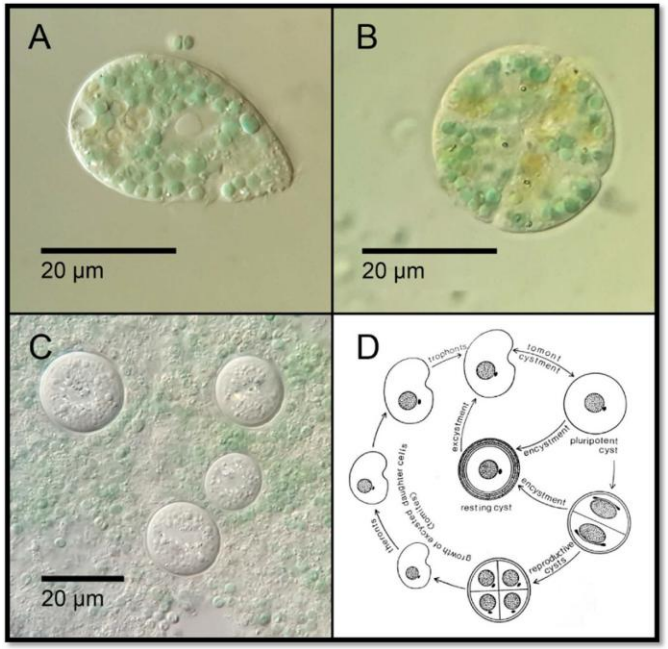


- It forms resting cysts.
- It can excyst fast when conditions become better.
- It is capable to clear a dense culture of *Synechocystis sp.* within 4-5 days.

Serious troubles with *Synechocystis salina* CCALA 192 culture scaling up to obtain biomass production.



Source: (Troschl et al.. 2017).

Tested ways of decontamination from *Colpoda steinii*

Table 2. Evaluation of different cultivating strategies against *Colpoda steinii* in large scale cultivation of Synechocystis salina CCALA192.

Cultivation strategy in the photobioreactor (flat panel – 100 L)	Outcome
Sanitizing with 70% ethanol solution	Culture crash 4–5 days after inoculation
Sanitizing with Dosyl 3 Plus solution	Culture crash 4–5 days after inoculation
Sanitizing with NaClO solution	Culture crash 4–5 days after inoculation
Partially anoxic conditions	No inhibition of *Colpoda steinii*. culture crash 4–5 days after inoculation
Use of filter combined with partially anoxic conditions	Culture crash 5–6 days after inoculation
High salinity (50 g/L NaCl)	Total inhibition of *Colpoda steinii* - Stable cultivation of *Synechocystis* CCALA192

How does high salinity affect *Colpoda steinii* in *Synechocystis* culture?

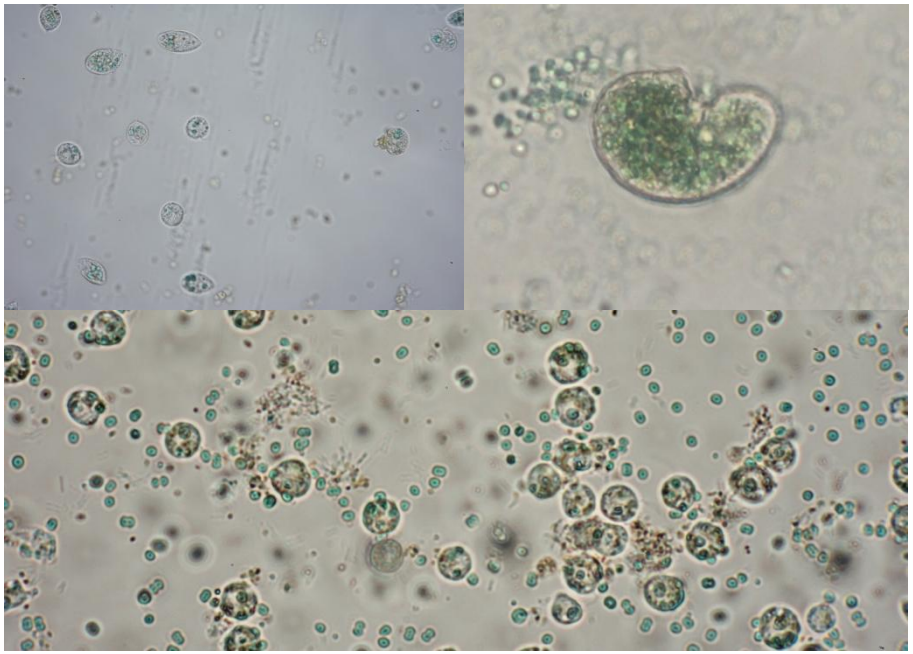


Fig. 1. Large scale cultivation of *Synechocystis salina* CCALA192 contaminated with *Colpoda steinii* (active mode).

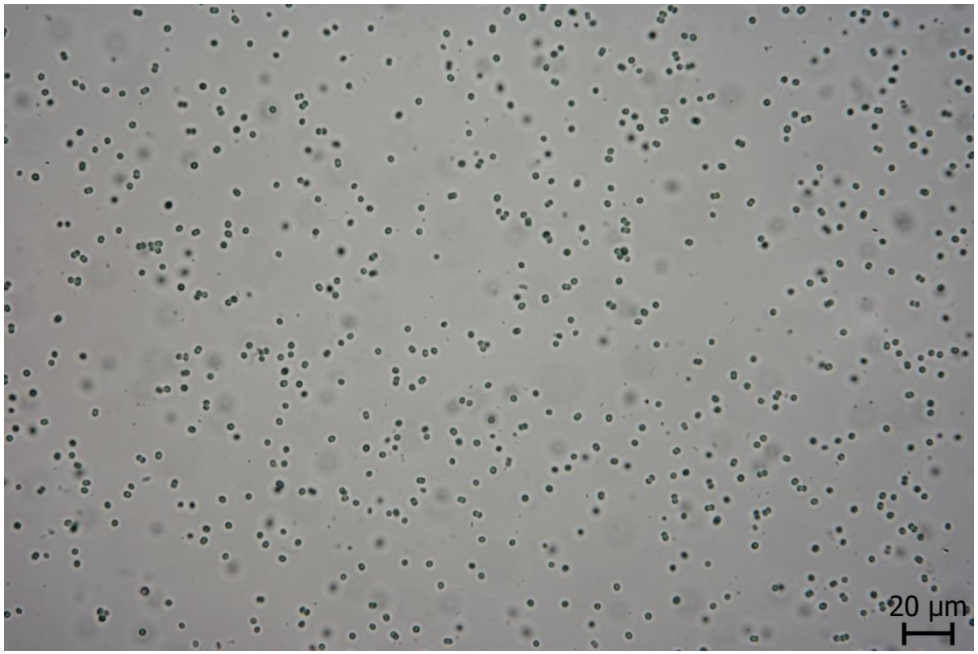
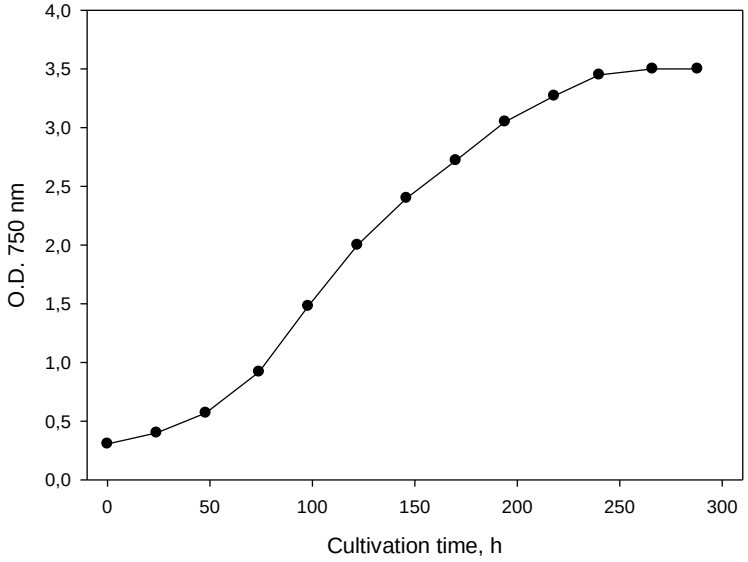


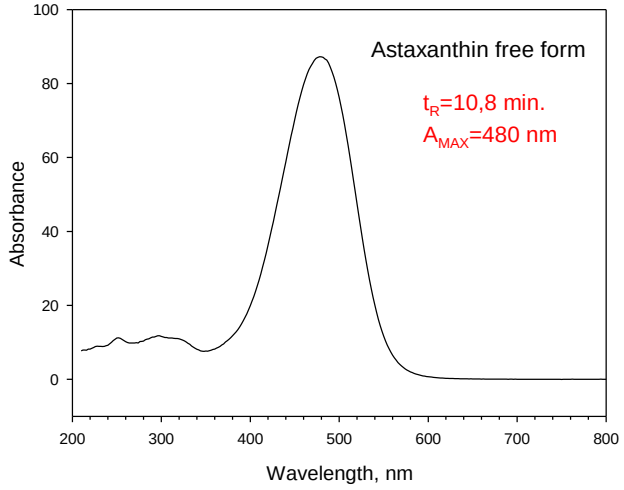
Fig. 2. Large scale cultivation of *Synechocystis salina* CCALA192 after high salt treatment (50 g/L).

Solution of our problem



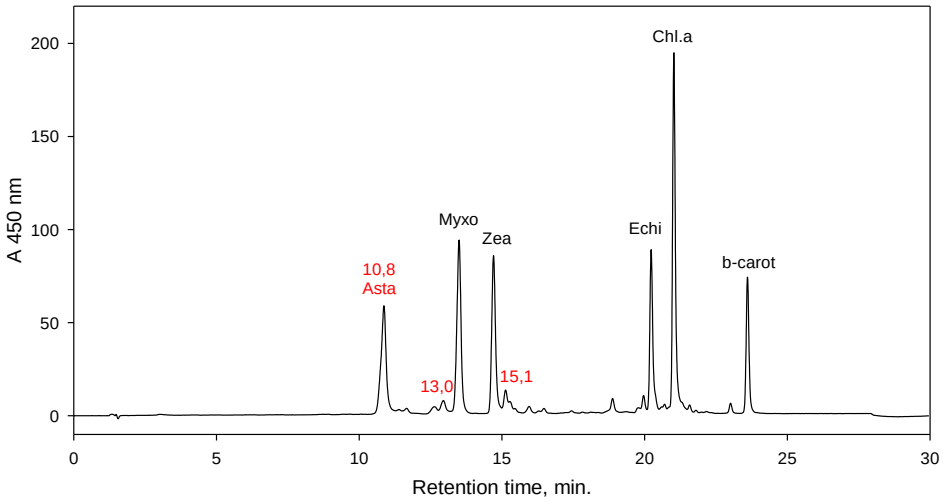
- Cultivation in 20 L incubator.
- Closed system

Culture started at 0.3 $O.D._{730}$ and grew up to O.D. 3.5. dry weight 1.1 g/L. which gained yield of 20 g in total (approx. 20 mg of astaxanthin).



Pigments of TiWZ cultivated in 20 L.

- Apart of the main pigments common in all *Synechocystis* strains. there were detected:



- Myxoxantophyll (t_R=13.5 min.)
- Zeaxanthin (t_R=14.6 min.)
- Echinenone (t_R=20.2 min.)
- Chlorophyll a (t_R=21.0 min.)
- β-carotene (t_R=23.6 min
- the free form of Astaxanthin eluting at t_R = 10.8 min
- two unknown peaks with characteristic spectra for secondary carotenoid (t_R=13.0 min. and t_R=15.1 min.). The content of the free form of Astaxanthin per dry biomass was **0.12%.**

Salt test

- Because TiWZ is a fresh water culture, we didn’t expect any tolerance to salt content in the medium.

Thank you for your attention

- Thank to our colleagues:
  Ing. Jiří Kopecký Ph.D.
  Elizabeth Figueroa M.Sc.

Mé otázky

- Jak byl identifikován Astaxanthin v obou peacích? 10.7 a 12.7?
- Jaký objem má menší kultivátor. který mi v prezentaci nasdílela Elizabeth? Je to 80 L?
- Jak se zbavili zlatých řas?

Tested ways of decontamination from *Colpoda steinii*

Table 1. Evaluation of different cultivating strategies against *Colpoda steinii* (Troschl et al.. 2017).

Cultivation strategy in the photobioreactor	Outcome
Sanitizing with 0.1% NaClO solution	Culture crash 4–5 days after inoculation
Sanitizing with 0.5% NH4 solution	Culture crash 4–5 days after inoculation
High salinity (20 g/L NaCl)	No inhibition of Colpoda steinii
Cultivating at pH 10	No inhibition of Colpoda steinii
High ammonium (200 mg/L $NH4^+$ at pH 8.5)	No inhibition of Colpoda steinii
CO_2 asphyxiation (262 mg/L dissolved CO_2 at pH 6.35)	No inhibition of Coploda steinii
Partially anoxic conditions	Stable cultivation of Synechocystis CCALA192

Source: (Troschl et al.. 2017).