Increasing Photoautotrophic Growth and Carotenoid Production with *Dunaliella Salina* .

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***Highlights:***

* Up to sevenfold increase of so far reported maximum biomass concentrations (5 gCDW L-1)
* Sixfold increase of β-carotene concentration with nutrient limitation at 18 % salinity (w/v)

# Introduction

The photoautotrophic production of high-value products from CO2 with microalgae is of increasing interest. *Dunaliella salina* is a halotolerant microalgae that can survive up to 30 % (w/v) NaCl thereby accumulating increasing amounts of β-carotene in the cells. Thus, *D. salina* is a highly interesting microalgae for outdoor cultivations in open photobioreactors with seawater as the salt content permanently increases due to the continuous evaporation of water. Unfortunately, the final biomass concentrations reported so far with *D. salina* are low compared to other microalgae.

# Methods

Growth and product formation of *D. salina* was studied in fully-controlled LED illuminated flat-plate gas-lift photobioreactors [1-3] with diurnal light (0 – 1900 µmol m‑2 s‑1) and temperature profiles (15 – 30 °C) according to [4] using CO2 for pH-control. Additionally, the salt content was increased from 3.5 % (w/v) up to 30 % (w/v) in order to get comparable data to outdoor cultivations with evaporation. Growth and β-carotene formation was studied independently. First, batch processes were performed using a growth medium. Second, *D. salina* was harvested and the cells were resuspended in a medium with reduced nutrient concentration. The salt content in this production phase was kept constant. Optical density, cell dry weight, carotenoid content and transmission were monitored in the batch processes.

# Results and discussion

Growth of *D. salina* in salt accumulating batch processes was comparable to the growth in batch processes with a constant salt concentration of 7.5 % NaCl. The final cell dry weight of 5 g L-1 (40 million cells mL-1) after 7.7 days was increased up to sevenfold compared to so far reported data [5–7]. Neither the variation of pH in a range of pH 7.5 – pH 8.5 nor an increase in temperature from 15 – 30 °C to 20 – 35 °C showed significant changes in biomass formation demonstrating robustness of *D. salina*. A maximum lutein concentration of 8.8 mg L-1 (2.0 mg g-1 cell dry weight) and a maximum β-carotene concentration of 5 mg L-1 (1.1 mg g-1 cell dry weight) were measured during growth with increasing salt concentrations. After transferring *D. salina* into a nutrient limited medium, highest β-carotene concentration could be observed in a batch process with a salinity of 18 % (w/v). The β-carotene concentration was increased sixfold from around 2 mg L-1 up to 12 mg L-1 (3.2 mg g-1 cell dry weight).

# Conclusion and outlook

The drawback of photoautotrophic processes with *D. salina* (low biomass concentrations) can be solved by changing the cultivation system from shaking flasks, tubular reactors or column reactors to fully-controlled LED illuminated flat-plate gas-lift photobioreactors which resulted in an enhancement of the final dry cell mass concentration by a factor of 7 compared to literature [5–7]. Applying nutrient limitation after growth resulted in an increase of the β-carotene concentration by a factor of 6. Scale-up of microalgae processes from lab-scale flat-plate gas-lift photobioreactors to open thin-layer cascade reactors with physical climate simulation [4] was already shown for several microalgae and will be approved for β-carotene production with *D. salina* in the future.

1. References

[1] Koller AP, Wolf L, Weuster-Botz D (2017): Reaction engineering analysis of *Scenedesmus ovalternus* in a flat-plate gas-lift photobioreactor. Biores Technol 225: 165–174.

[2] Koller A, Loewe H, Schmid V, Mundt S, Weuster-Botz D (2016) Model-supported phototrophic growth studies with *Scenedesmus obtusiusculus* in a flat-plate photobioreactor. Biotechnol Bioeng 114: 308–320.

[3] Pfaffinger CE, Schöne D, Trunz S, Loewe H, Weuster-Botz D (2016): Model-based optimization of microalgae areal productivity in flat-plate gas-lift photobioreactors. Algae Res 20: 153–163.

[4] Apel AC, Pfaffinger CE, Basedahl N, Mittwollen N, Goebel J, Sauter J, Brueck T, Weuster-Botz D (2017): Open thin-layer cascade reactors for saline microalgae production evaluated in a physically simulated Mediterranean summer climate. Algal Research 25: 381–390.

[5] Wu Z, Duangmanee P, Zhao P, Juntawong N, Ma C (2016): The Effects of Light, Temperature, and Nutrition on Growth and Pigment Accumulation of Three *Dunaliella salina* Strains Isolated from Saline Soil, Jundishapur journal of microbiology 9 e26732.

[6] Prieto A, Pedro Cañavate J, García-González M (2011): Assessment of carotenoid production by *Dunaliella salina* in different culture systems and operation regimes. Journal of Biotechnology 151: 180–185.

[7] Morowvat MH, Ghasemi Y (2016): Culture medium optimization for enhanced β-carotene and biomass production by *Dunaliella salina* in mixotrophic culture. Biocatalysis and Agricultural Biotechnology 7: 217–223.

[8] Marín N, Morales F, Lodeiros C, Tamigneaux E (1998): Effect of nitrate concentration on growth and pigment synthesis of *Dunaliella salina* cultivated under low illumination and preadapted to different salinities. Journal of Applied Phycology 10: 405–411.