

CellMaker Application Note:

Cultivation of *Dunaliella salina* and *bardawil* using a novel Single-Use Bioreactor (SUB) for triglyceride and glycerol production

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Introduction

Dunaliella is a unicellular, biflagellate, photoautotrophic green microalga found in extreme, salt-water conditions around the globe. *Dunaliella* is currently exploited at commercial scale for the production of carotenoids.

Eukaryotic cells accumulate neutral lipids (triacylglycerides, TAG) in lipid droplets surrounded by a sterol-coated phospholipid monolayer (Zweytick et al., 2000). *Dunaliella* is not known to accumulate TAG but will do so in the presence of an electron transport chain inhibitor such as azide (Zalogin and Pick, 2014), or under conditions of oxidative stress induced with H₂O₂ (Yilancioglu et al, 2014).

In this study, *Dunaliella salina* and *bardawil* were cultivated in a novel Single-Use Bioreactor (SUB) (Fig. 1) in the presence of azide to assess its effects on the production not only of TAG, but also carotenoids and glycerol.

The SUB uses the principle of airlift: bubbles are sparged through the culture medium in a disposable bag. We set up a simple lighting rig to provide a 12 h light, 12 h dark cycle at the light intensity required, and sparged the unit with CO₂ instead of O₂, to maintain a constant supply of CO₂. We compared the growth of two strains *Dunaliella* in the Cellmaker with flask-grown cells grown in a growth cabinet under the same conditions of light intensity and CO₂ concentration.



Fig. 1
Cellexus SUB showing cultivation of *Dunaliella* (green bag) compared to flask-grown *Dunaliella*.

Results

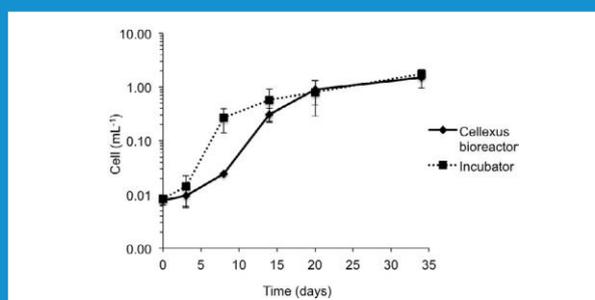


Fig. 2
Growth curves for cells cultured in flasks in the incubator (dotted line) and in the Cellexus SUB (Solid line). The cell doubling time in the SUB was slight lower for cells grown in flasks. The reason is unknown but may reflect a physiological trait of this biflagellate alga.

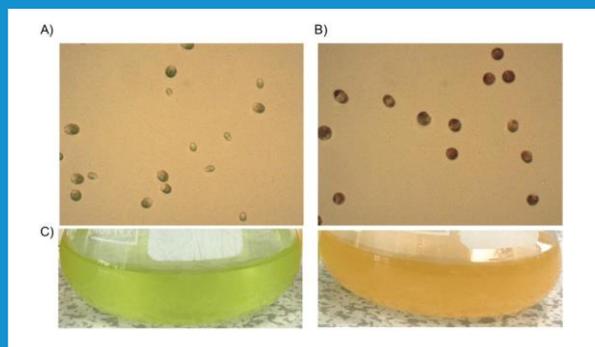


Fig. 3
Morphology of cells cultivated in Cellexus SUB (A) and incubator (B). Chlorophyll and carotenoid contents for strain 1 were 2.8 & 2.2 fold greater in the SUB compared to flask-grown cells, but lower for strain 2, reflecting differences in their light requirements.

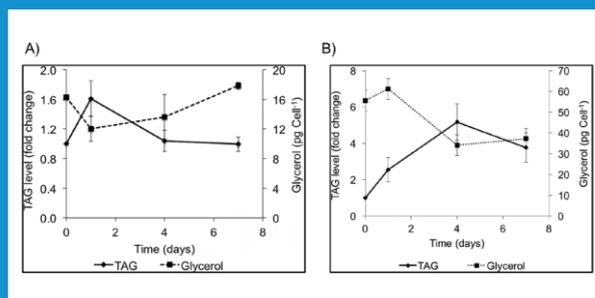


Fig. 4
Effect of azide on triglyceride (TAG) and glycerol accumulation. A) Cellexus SUB, B) Incubator. TAG induction was monitored with Nile red staining. Data were normalised to azide untreated sample (day 0), and plotted as a fold change of TAG accumulation. Glycerol content was 1.2-fold greater for strain 1 cells grown in the Cellexus SUB, but less for strain 2. Glycerol appeared at the expense of lipid breakdown.

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Conclusions

- 1) We were able to use the Cellmaker to successfully cultivate *Dunaliella* in the presence of a metabolic inhibitor to increase the yield of glycerol and carotenoids and dispose of the bag after use.
- 2) Carotenoids and chlorophyll production varied between strains, attributed to their differing light requirements to trigger carotenogenesis. These differences warrant investment in a more sophisticated lighting system.
- 3) Glycerol and lipid contents varied between strains, reflecting differences in response to azide.
- 4) Since the bags are disposable, problems of cell adhesion and biofilm production that would otherwise have reduced light penetration to the cells were not encountered in the Cellmaker. This is in contrast to the situation with the use glass vessels in which cultures frequently crash with time, when the vessels are reused.
- 5) *Dunaliella salina* and *bardawil* were successfully cultivated in the Cellexus Cellmaker under the harsh stress-related growth conditions required by this extremophile to produce high value carotenoids.

References

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- Zalogin TR, Pick U (2014 b) Inhibition of nitrate reductase by azide in microalgae results in triglycerides accumulation. *Algal Research* 3: 17-23
- Zweytick D, Athenstaedt K, Daum G (2000) Intracellular lipid particles of eukaryotic cells. *Biochimica et biophysica acta* 1469: 101-120

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