Harvesting of the Fragile Alga *Dunaliella salina* by Membrane Filtration with Permeate Recovery for Microalgae Cultivation

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ONE FINE DAY IN THE LAB...

BY LEONID SCHNEIDER

WITH ALL THESE MANY AUTHORS WE HAD ON OUR PAPER... NOBODY KNEW YOU WERE A DOG!
Harvesting of *Dunaliella salina* Keeping Cell Integrity

- **Pre-concentration of biomass by membrane processing prior to centrifugation**
  - Reduce overall energy and capital costs
  - Maximise volumetric fluxes while maintaining cell integrity at maximised values of concentration factors

Membrane units

Low-shear centrifuge (Spiral Plate Technology, Evodos)
Harvesting of *Dunaliella salina* by Membrane Filtration

- **Harvesting of *Dunaliella salina* in low carotenogenic state ("green"):** operation under controlled transmembrane pressure

**Membrane Characteristics**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Membrane area (m²)</td>
<td>2.4</td>
</tr>
<tr>
<td>Number of fibers</td>
<td>832</td>
</tr>
<tr>
<td>Material</td>
<td>Polyethersulfone</td>
</tr>
<tr>
<td>MWCO</td>
<td>150 kDa; 0.1 µm</td>
</tr>
</tbody>
</table>

**Parameters monitored:** permeate flux; feed and permeate pressures; optical microscope observation; cell integrity measured by % cell viability (Muse® Cell Analyser)

No pre-filter
Flexible impeller pump
Harvesting of *Dunaliella salina* by Membrane Filtration

- **Optimized conditions** (from the single batch experiments): ultrafiltration system operated with a $v_{\text{cross-flow}}$ of 0.6 m/s; transmembrane pressure of 0.25 bar; backflush each 20 minutes

\[
\text{Cf}_{\text{overall}} = 16.4 \text{ (or 94% permeate recovery)} \\
J_v \text{ average} = 22 \text{ L/(m}^2\text{.h)} \\
\text{Cell integrity loss} = 13\%
\]
Harvesting of *Dunaliella salina* by Membrane Filtration

- **Harvesting of *Dunaliella salina* in carotenogenic phase ("orange"):** operation under controlled permeate flux

![Diagram of membrane filtration process]

**Parameters monitored:** permeate flux; feed and permeate pressures; optical microscope observation; cell integrity measured by % cell viability (Muse® Cell Analyser)

**Membrane characteristics**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Membrane area (m²)</td>
<td>0.48</td>
</tr>
<tr>
<td>Number of fibers</td>
<td>520</td>
</tr>
<tr>
<td>Material</td>
<td>Polysulfone</td>
</tr>
<tr>
<td>MWCO (kDa)</td>
<td>100, 500</td>
</tr>
</tbody>
</table>

No feed restriction valve
Harvesting of *Dunaliella salina* by Membrane Filtration

- **Consecutive batches (3)** - with single batch optimized operating conditions
- **Optimized conditions** - MWCO 100 kDa; \( v_{\text{cross-flow}} \) of 0.35 m/s; \( J_v \) = 10 L/m\(^2\).h and backflush cycles of 9 min permeation + 1 min relaxation

\[
\text{Cf}_{\text{overall}} = 36.7 \text{ (or 97% permeate recovery)} \\
J_v \text{ average} = 10 \text{ L/(m}^2\text{.h)} \\
\text{Cell integrity loss} = 6.9\%
\]
Harvesting of *Dunaliella salina* by Membrane Filtration Followed by Evodos Centrifugation

Energy consumption (membrane unit) = 0.87 kWh/m$^3$

Energy consumption (centrifuge) = 2.5 kWh/m$^3$

**Reduction of the OPEX + CAPEX** of 52% and **reduction of energy consumption** of 45% when applying a two-step approach with pre-concentration (by ultrafiltration), when comparing with a stand-alone harvesting by centrifugation

Monitoring of *Dunaliella salina* by 2D Fluorescence Spectroscopy

- Highly sensitive
- Non-invasive and non-destructive
- An optical fibre can be used *in situ* and *online*
- Detects the presence of natural fluorophores in nature:
  - Proteins (I)
  - Humic compounds (II)
  - Pigments (III)

**EEM** = excitation/emission matrix

(excitation wavelength, emission wavelength, intensity of emission)
Monitoring of *Dunaliella salina* by 2D Fluorescence Spectroscopy

Several interferences in complex biological media prevents direct spectra interpretation. Interactions between molecular species are valuable information if properly extracted!

Principal Component Analysis (PCA)

Projection to Latent Structures (PLS)

\[ a_1x_1 + b_1x_2 + c_1x_3 + d_1x_4 + ... = y_1 \]
\[ a_2x_1 + b_2x_2 + c_2x_3 + d_2x_4 + ... = y_2 \]

find correlations to predict cellular growth and other key performance parameters

M. Sá, J. Monte, C. Brazinha, C.F. Galinha, J.G. Crespo,
2D Fluorescence spectroscopy for monitoring *Dunaliella salina* concentration and integrity during membrane harvesting,
Harvesting of “green” *Dunaliella salina* DF17:

**Cell Number**

Train.: $R^2 = 0.86$, Slope = 1.00  
Valid.: $R^2 = 0.83$, Slope = 0.78  
RMSEP = $1.3 \times 10^5$ cells/mL

**Viability (%)**

Train.: $R^2 = 0.91$, Slope = 1.00  
Valid.: $R^2 = 0.82$, Slope = 0.70  
RMSEP = 5.3% viability
Harvesting of “orange” *Dunaliella salina* DF40:

- **Cell Number**
- **%Viability**

### Observed vs Predicted Values

#### Train:
- $R^2 = 0.93$, Slope = 1.00
- RMSEP = 1.4E+6 cells/mL

#### Valid.:
- $R^2 = 0.93$, Slope = 1.15
- RMSEP = 10% viability

**Cell Conc. (cells/mL) vs Observed**

**Viability (%) vs Observed**
Dunaliella salina Culture Medium Recycling after Membrane Harvesting

• Permeate treatment for culture medium recycling
  o From a consecutive batch harvesting experiment processing 40 L of microalgae culture

• With subsequent treatment using advanced oxidation techniques
  o UV + H₂O₂
  o Ozone + H₂O₂

![Graph showing Glycerol content vs time for UV+H₂O₂ and Ozono+H₂O₂ treatments.](image1)

![Graph showing Nitrites & Nitrates vs time for Ozono+H₂O₂ and UV+H₂O₂ treatments.](image2)
Culture medium recycling was tested in lab conditions, in bubble columns, under constant 300 µmol.m².s⁻¹. Semi-continuous regime, in orange (carotenogenic) growth stage.

Using O₃+H₂O₂ did not allow correct control of the nitrogen concentration (culture is greenish), which is key for carotenogenesis.
Dunaliella salina Culture Medium Recycling after Membrane Harvesting

The untreated permeate and permeate treated with UV+H2O2 provided better results than the control.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Maximum Volumetric Productivity of Carotenoids (mg Car/L/d)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Permeate without treatment</td>
<td>6.93 ± 0.48</td>
</tr>
<tr>
<td>Permeate + UV + H2O2</td>
<td>5.98 ± 0.41</td>
</tr>
</tbody>
</table>
**Dunaliella salina** Culture Medium Recycling after Membrane Harvesting

- In spite of the presence of bacteria, *Dunaliella* growth and carotenogenesis were possible in all cultures. Quite successful in 2 cases!

Preliminary results, so more improvements will come:

- Mineral composition of the 4 culture media at the end of the 3rd cycle still pending
- Culture medium optimization is ongoing and directly influences efficiency of the treatment + recycling strategy
Harvesting of the Fragile Alga *Dunaliella salina*

Development of a prototype Evodos50 (automated) dynamic settler
Evodos Dynamic Settlers

- Algae harvesting (small and fragile micro algae)
Evodos Technology

- Path of a particle in rotating fluid cylinder

1. Rotating water cylinder, cross-section.
2. Spiral plates limit path of particle.
3. 90, 135 or 180 SPT vanes hinged to main shaft.
4. Plate pack detail. Max. swimming distance = 7 mm.

\[ F_c = 2000 - 4500 \times G \]
Evodos Technology

“Evodos redefines separation by accelerating nature’s laws through the process of Thin Layer Laminar Settling, using spiral plate technology®.”
Evodos Technology

Small particles will settle on the spiral plates due to the g-forces in combination with the small travel distance (distance between the plates, 7 mm.)

Discharge cycle to separate the paste from the spiral plates
Evodos 10 harvesting results (initial tests, pilot machine)

<table>
<thead>
<tr>
<th></th>
<th>liters/hour</th>
<th>Intact cells harvested</th>
<th>dry weight content</th>
</tr>
</thead>
<tbody>
<tr>
<td>Evodos 10</td>
<td>250</td>
<td>Yes, &gt; 95%</td>
<td>20-30 %</td>
</tr>
<tr>
<td>Evodos 10</td>
<td>300</td>
<td>Yes, &gt; 95%</td>
<td>20-30 %</td>
</tr>
<tr>
<td>Max. capacity</td>
<td>350 liters/hour</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Evodos 50 development

Automated machine developed for capacities up to 4000 liters/hour
Evodos set-up for harvesting

Evodos50 harvest *Dunaliella* intact algae(!) in paste of 40% solids(!) and high beta-carotene to above 10% AFDW (!).

Stacked disc centrifuge harvest of the same culture broken *Dunaliella* in paste of 12% solids and beta-carotene of below 8%.

**Evodos 50:**
- 4000 litre/hour
- 95% separation efficiency
- >20 hours/day
Evodos 50 test results
Evodos 50 test results

Best results achieved

Test overview
Feed 0,1 – 1 gram /litre

<table>
<thead>
<tr>
<th>Date</th>
<th>Harvester</th>
<th>Pond nr.</th>
<th>Setting speed (RPM)</th>
<th>Setting pump (litres/hour)</th>
<th>Input (+/- m³)</th>
<th>Paste (litres)</th>
<th>Solids (%)</th>
<th>Expected car. (%)</th>
<th>Efficiency (%)</th>
<th>Cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>13-Dec</td>
<td>Evodos 50A</td>
<td>25</td>
<td>4200</td>
<td>4000</td>
<td>4</td>
<td>2,5</td>
<td>44</td>
<td>1,8</td>
<td>95</td>
<td>INTACT</td>
</tr>
<tr>
<td>14-Dec</td>
<td>Evodos 50A</td>
<td>26</td>
<td>3000</td>
<td>3000</td>
<td>3</td>
<td>2</td>
<td>37,7</td>
<td>2,18</td>
<td>93</td>
<td>INTACT</td>
</tr>
<tr>
<td>15-Dec</td>
<td>Evodos 50A</td>
<td>1</td>
<td>2000</td>
<td>2000</td>
<td>2</td>
<td>1,25</td>
<td>24</td>
<td>1,76</td>
<td>95</td>
<td>INTACT</td>
</tr>
</tbody>
</table>

Total batch, 120,000 litre, Average energy consumption 1,10 kWh / 1000 litre input

Future steps:
Harvesting whole year round to check the performance in different seasons and check the quality of the paste/ components in the biorefinery step.